3 Secondary Models

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CONTENTS

3.1 INTRODUCTION

Changes in populations of microorganisms in foods over time (i.e., "microbial kinetics") are governed by storage conditions ("extrinsic" factors) and product characteristics ("intrinsic" factors). Collectively these have been termed "environmental parameters." They may represent simple situations, e.g., where the storage temperature is the only important factor influencing microbial kinetics, but in many foods the environmental parameters that influence microbial kinetics are complex and dynamic and include the combined effects of extrinsic factors such as temperature and storage atmosphere; intrinsic factors such as water activity, pH, naturally occurring organic acids, and added preservatives; and interactions between groups of microorganisms.

Consistent with the widely accepted terminology introduced by Whiting and Buchanan (1993), we term those models that describe the response of microorganisms to a single set of conditions *over time* as "primary" models (see Chapter 2). Models that describe the effect of environmental conditions, e.g., physical, chemical, and biotic features, on the values of the parameters of a primary model are termed "secondary" models.

Knowledge of the environmental parameters that most in uence growth of microorganisms in foods is essential for the development, as well as for the practical use, of predictive microbiology models. Secondary models that do not include all the environmental parameters important in a food are said to be "incomplete" (Ross, Baranyi, McMeekin, 2000) and require expansion (or simple "calibration" if those factors are constant) to accommodate their effect on microbial kinetics. The environmental parameters that are important for particular foods, however, are not always known. In those situations the systematic approach of predictive microbiology can help to elucidate the microbial ecology of the product.

In this chapter we consider a range of types of secondary models including those that model the probability that a predicted kinetic response will occur. The chapter includes descriptions and comparison of models, as considerations for development of robust, secondary models. Appendix A3.1 details methods to measure environmental factors of importance — an essential element of the application of predictive microbiology.

3.1.1 Philosophy, Terminology, and Methodology

The history of predictive microbiology, including the philosophical motivations of Roberts and Jarvis (1983), who first proposed the concept, w as traced by Ross and McMeekin (1994). From a purely pragmatic perspective, predictive microbiology aims to collect and make accessible computerized data on the behavior of microbial populations in response to defined environmental conditions, but mathematical modeling also provides a useful and rigorous framework for the hypothetico-deductive scientific process. To develop a consistent framework that enables us to understand and predict the microbial ecology of foods it is desirable to integrate the patterns of microbial behavior revealed in predictive modeling studies with knowledge of the physiology of microorganisms and physical and chemical processes and phenomena that occur in foods and food processes (Ross, Baranyi, McMeekin, 2000).

Various types and categorizations of models are recognized. Empirical models are, essentially, pragmatic and simply describe a set of data in a convenient mathematical relationship with no consideration of underlying phenomena. Mechanistic models are built up from theoretical bases and, if they are correctly formulated, can allow the response to be interpreted in terms of known physical, chemical, and biological phenomena. An advantage of mechanistic approaches is that they tend to provide a better foundation for subsequent development and expansion of models; i.e., taken to their logical extreme, models for specific situat ions would simply be special, or reduced, cases of a much larger and holistic model that describes, quantitatively, the microbial ecology of foods. The process of developing models that are able to be integrated readily with other models so as to describe more complex phenomena has been termed "nesting" or "embedding." A fuller explanation of the bene ts of that approach w as provided by Baranyi and Roberts (1995).

In one sense, a model is the mathematical expression of a hypothesis. If this approach is adopted, it follows that the parameters in such models might be readily interpretable properties of the system under study, and that the mathematical form of the model would enable interpretation of the interactions between those factors. Interpretability of model parameters is a feature highly valued by many authors in the predictive microbiology literature (e.g., Augustin and Carlier, 2000a,b; Rosso et al., 1993; Wijtzes et al., 1995). Although the development of predictive microbiology has seen the embedding of more and more mechanistic elements, or at least models whose structure and parameterization reflects known or hypothesized underlying phenomena, in practice many models currently available in predictive microbiology are not purely empirical, and none are purely mechanistic (Ross, Baranyi, McMeekin, 2000).

Another, often cited, advantage of mechanistic models is that if they are built on sound theory they are more likely to facilitate prediction by extrapolation. Conversely,

as none of the models in use in predictive microbiology can be considered to be mechanistic, they can only be used to make predictions by interpolation. (Determination of the interpolation region encompassed by a model is discussed in Sections 3.2.5 and 3.4.3.4.) It is perhaps ironic, then, that 20 years of experience in predictive microbiology has not demonstrated the practical usefulness of mechanistic models that have been proposed to date (see Section 3.2.4). In general, even with good quality data the mechanistic models do not provide better fit and are us ually harder to work with than quasi-mechanistic or empirical models currently used.

Predictive microbiology is a specific application of the field of mathematical modeling and, as such, the same rules of modeling as are applied in those other disciplines are relevant to the development of predictive food microbiology models. These have been discussed by various authors (Draper and Smith, 1981; McMeekin et al., 1993; Ratkowsky, 1993), and an overview is presented in Table 3.1.

Experimental methods and design considerations relevant to kinetic models were discussed in detail in McMeekin et al. (1993; Chapter 2), Davies (1993), and Legan et al. (2002) and are also discussed in Chapter 1. Two points that we feel are necessary to reiterate are the limitations of the central composite design in predictive microbiology studies, and consideration of spoilage domains when growth of spoilage microorganisms is studied. Legan et al. (2002) accentuated the importance of experimental design in growth modeling studies stating:

> in other disciplines, such as engineering, central composite designs are commonly used for developing response surface models. For microbiological modeling, however, these designs have serious limitations and should be avoided. Central composite designs concentrate treatments in the centre of the design space and have fewer treatments in the extreme regions where biological systems tend to exhibit much greater variability.

Microbial food spoilage is dynamic and in some cases relatively small changes in environmental parameters cause a complete shift in the micro or a responsible for product spoilage. Thus, to avoid modeling growth of spoilage microorganisms under conditions where they have no in uence on quality , a product-oriented approach that includes determination of the spoilage domain of specific micoor ganisms is often required (Dalgaard, 2002).

We will not comment further on methodology appropriate to development of kinetic models, other than to say that to develop reliable secondary models an understanding of microbial physiology and its interaction with food environments and storage and processing conditions must be borne in mind in the design of experiments including the preparation of cultures and interpretation of measurements of population changes. This issue is particularly explored and exempli ed in Section 3 .4.4.4 concerning experimental considerations relevant to the development of growth limits models.

3.2 SECONDARY MODELS FOR GROWTH RATE AND LAG TIME

Implicit in the appropriate development and use of secondary models in predictive food microbiology is the ability to characterize foods, and the environment they

TABLE 3.1
Some Considerations in the Selection of Models

Subject	Reasons
Parameter estimation properties	Relates to the procedure and reliability of estimating the model parameters. In general, models should have parameters that are independent, identically distributed, normal or "iidn" (see, e.g., Ratkowsky, 1993)
Stochastic assumption	The form of the model, and choice of response variables, should be such that the difference between prediction and observations (or some mathematical transformation of them) is normally distributed, and that the magnitude of the error is independent of the magnitude of the response modeled. If not, the fitting can be dominated by some data, at the expense of other data
Parameter interpretability	As noted in the text, it is useful if the parameters have biological/physical/chemical interpretations that can be readily related to the independent and dependent variables. This can simplify the process of model creation and also aid in understanding of the model (This may be less important than the behavior and performance of the model.)
Parsimony	Models should have no more parameters than are required to describe the underlying behavior studied. Too many parameters can lead to a model that ts the *error* in the data, i.e., generates a model that is specific to a particular set of observations. Nonparsimonious models have poor predictive ability
Interpolation region	No models in predictive microbiology can be considered to be mechanistic and predictions can be made by interpolation only. Thus, the interpolation region de nes the useful range of applicability of the model. The interpolation region is affected by not only the range of individual variables, but also the experimental design (see Section 3.2.5)
Correct qualitative features	In mathematical terms, these are the analytical properties of the model function. They include convexity, monotonity, locations of extreme, and zero values. If biological considerations prescribe any of these, the model should reflect those properties accurately
"Extendibility" (embedding, nesting)	When a model is developed further (such as to include more or dynamically changing environmental factors) the new, more complex model should embody the old, simpler model as a special case

Source: Modi ed from Ross, T., Baranyi, J., and McMeekin, T.A. In *Encyclopaedia of Food Microbiology*, Robinson, R., Batt, C.A., and Patel, P. (Eds.), Academic Press, London, 2000, pp. 1699–1710.

present to contaminating microorganisms, in terms of those biotic and abiotic elements that affect the dynamics of the microbial population of interest. Methods to characterize the physicochemical environment, including temperature, gaseous atmosphere, salt and/or water activity, pH and organic acids, spices, smoke, and other components, are discussed in detail in Appendix A3.1. These topics are also considered in Chapter 5, including discussion of the in uence o f other organisms and heterogeneity in the environment. Another important element is the ability to characterize temporal changes in the environment. Techniques for modeling microbial population dynamics under time-varying conditions are considered in Chapter 7.

Within predictive microbiology the development and application of secondary models for growth rates and lag times have been extensively reviewed (Buchanan, 1993b; Davey, 1999; Farber, 1986 ; ICMSF, 1996a,b; McDonald and Sun, 1999; McMeekin et al., 1993; Ross, 1999a,b; Ross and McMeekin, 1994; Skinner et al., 1994; Whiting, 1995). This section describes types of secondary growth rate and lag time models that are currently available, but with particular focus on more recent developments, and also includes a detailed tabulation of models available for specific microorganisms.

3.2.1 Square-Root-Type Models

3.2.1.1 Temperature

As discussed later (Section 3.2.4), in many cases the classical Arrhenius equation is inappropriate to describe the effect of suboptimal temperature on growth rates of microorganisms because the (apparent) activation energy (E_a) itself is temperature dependent. To overcome this problem Ratkowsky et al. (1982) suggested a simple empirical model (Equation 3.1). When this model was fitted to experimental growth rates the data were square-root transformed to stabilize their variance and this simple model and its numerous expansions are named square-root-type, Ratkowsky-type, or Bêlerádek-type models (McMeekin et al., 1993). These models, and the closely related cardinal parameter models (see Section 3.2.3), are probably the most important group of the secondary models within predictive microbiology.

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \tag{3.1}$$

where b is a constant and T is the temperature. The parameter T_{min}, a theoretical minimum temperature for growth, is the intercept between the model and the temperature axis (Figure 3.1). T_{min} is a model parameter and its value can be 5 to 10°C lower than the lowest temperature at which growth is actually observed. This interpretation differs from that embodied in the cardinal parameter models, as discussed in Section 3.2.3 and Chapter 4).

From growth rates measured at several different constant temperatures the values of b and T_{min} in Equation 3.1 can be determined by classical model tting techniques (see Chapter 4). Recently it was suggested that b and T_{min} could be estimated from a single, optimally designed, experiment where growth resulting from a temperature profile is recorded (Bernaerts et al., 2000). These authors concluded that such an optimal, dynamic, one-step experiment would reduce the experimental work required to develop a model signi cantly and would have substantial potential within predictive microbiology. So far this technique has not found wider use within predictive microbiology and its ability to estimate model parameters accurately remains to be con rmed for different microorganisms and environmental parameters.

Ratkowsky et al. (1983) expanded Equation 3.1 to include the entire biokinetic range of growth temperatures (Equation 3.2, Figure 3.1). From this model the optimal

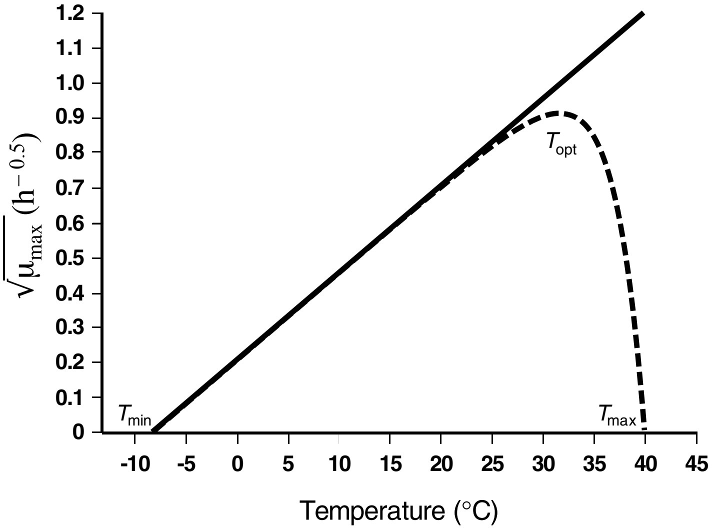


FIGURE 3.1 Simulation of Equation 3.1 (solid line) and Equation 3.2 (dashed line). b = 0.025 $h^{0.5}$/°C, T_{min} = –8°C, c = 0.30°C^{-1}, and T_{max} = 40°C.

growth temperature can be determined by solving the following equation: $c \times (T_{opt} - T_{min}) = \exp[c \times (T_{opt} - T_{min})] - 1$ (McMeekin et al., 1993).

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot (1 - \exp(c(T - T_{max}))) \tag{3.2}$$

where b and c are constants, T is the temperature, T_{min} the theoretical minimum temperature below which no growth is possible, and T_{max} is the theoretical maximum temperature beyond which growth is not possible.

While Ratkowsky et al. (1982, 1983) settled for an exponent of 2, the original Bêlerádek models had a variable exponent value. Dantigny (1998) and Dantigny and Molin (2000) used the concepts of dimensionless growth rate variables (effectively the same as the gamma factor concept; see Section 3.2.3) to explore the most appropriate value of the exponent for bacterial growth rate data using Bêlerádek-type models. They reported a correlation between the estimate of T_{min} and the exponent value used and found that when T_{min} and the exponent were simultaneously fitted by nonlinear re gression, thermophiles had lower fitted e xponent values than did mesophiles or psychrotrophic organisms. They reported that the use of the square-root model leads to an underestimation of the minimum temperature for growth when the exponent value is significantly less than 2.

3.2.1.2 Water Activity

McMeekin et al. (1987) found that growth responses of *Staphylococcus xylosus* followed Equation 3.1 at different values of water activity. T_{min} was constant and

thus independent of water activity and Equation 3.3 was suggested to describe the combined effect of temperature and water activity (McMeekin et al., 1987).

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot \sqrt{a_w - a_{w\,min}} \tag{3.3}$$

where b and T_{min} are as previously de ned, a_w is the water activity, and $a_{w\,min}$ is the theoretical minimum water activity below which growth is not possible.

Later, Miles et al. (1997) suggested that Equation 3.4 be used to take into account the effect of the entire biokinetic ranges of both temperature and water activity.

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot (1 - \exp(c(T - T_{max}))) \cdot \sqrt{(a_w - a_{w\,min})(1 - \exp(d(a_w - a_{w\,max})))} \tag{3.4}$$

where b, c, T, T_{min}, T_{max}, a_w, and $a_{w\,min}$ are as previously defined, d is a fitted constant, and $a_{w\,max}$ is a theoretical maximum water activity beyond which growth is not possible.

Most food-related microorganisms grow at water activities very close to 1.000 and in those cases the expanded water activity term (i.e., containing $a_{w\,max}$) in Equation 3.4 is not needed to predict growth in foods. However, some microorganisms, e.g., several marine bacteria, have a substantial requirement for minerals. To model growth responses of these microorganisms, the inhibitory effect of high water activities, i.e., low salt concentrations, must be taken into account. For the human pathogen *Vibrio parahaemolyticus,* $a_{w\,max}$ has been determined to be 0.998. Some seafood spoilage bacteria are more inhibited by high water activity; e.g., growth of *Halobacterium salinarium* was only observed at a_w values below 0.9 (Chandler and McMeekin, 1989; Doe and Heruwati, 1988; Miles et al., 1997).

3.2.1.3 pH

For *Yersinia enterocolitica,* Adams et al. (1991) found that growth responses followed Equation 3.1 at different values of pH. Again, T_{min} was constant and Equation 3.5 was suggested.

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot \sqrt{pH - pH_{min}} \tag{3.5}$$

where pH_{min} is the theoretical minimum pH below which growth is not possible and other parameters are as previously defined.

On the basis of the observation of Cole et al. (1990) that growth rate was proportional to hydrogen ion concentration, Presser et al. (1997) introduced the following quasi-mechanistic term to describe the effect of pH on bacterial growth:

$$\mu_{\max} = \mu_{opt} \times (1 - 10^{pH_{\min} - pH}) \tag{3.6a}$$

By analogy, another term was introduced for superoptimal (i.e., alkaline) pH conditions, leading to the following model for the entire biokinetic pH range:

$$\mu_{\max} = \mu_{opt} \times (1 - 10^{pH_{\min} - pH}) \times (1 - 10^{pH - pH_{\max}}) \tag{3.6b}$$

The validity of that term was evaluated against an extensive data set for *Escherichia coli* growth, including variables of temperature, water activity, and lactic acid concentration for a range of acid and alkaline environmental pH levels (see Equation 3.10).

Wijtzes et al. (1995, 2001) continued the development of square-root-type models and suggested Equation 3.7 for growth responses of *Lactobacillus curvatus* at different temperatures, a_w values, and pH

$$\mu = b \cdot (a_w - a_{w\,\min}) \cdot (pH - pH_{\min}) \cdot (pH - pH_{\max}) \cdot (T - T_{\min})^2 \tag{3.7}$$

3.2.1.4 Other Factors

Equation 3.8 was suggested to model the effect of carbon dioxide-enriched (%CO_2) atmospheres on growth of the specific spoilage organism *Photobacterium phosphoreum* on sh (Dalgaard, 1995; Dalgaard et al., 1997). Later, similar but square-root-transformed terms were used to model the effect of CO_2 and sodium lactate (NaL) on growth of *Lactobacillus sake* and *Listeria monocytogenes* at a constant pH (Equation 3.9; Devlieghere et al., 1998, 2000a,b, 2001).

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \times \frac{(\%CO_{2\,\max} - \%CO_2)}{\%CO_{2\,\max}} \tag{3.8}$$

$$\begin{aligned} \sqrt{\mu_{\max}} &= b \\ &\cdot (T - T_{\min}) \\ &\cdot \sqrt{a_w - a_{w\,\min}} \\ &\cdot \sqrt{CO_{2\,\max} - CO_2} \\ &\cdot \sqrt{NaL_{\max} - NaL} \end{aligned} \tag{3.9}$$

As noted above, a more comprehensive square-root-type model that includes the effects of temperature, pH, water activity, and lactic acid has been suggested and developed in a series of publications (Presser et al., 1997; Ross, 1993a,b; Salter et al., 1998; Tienungoon, 1988) and has been applied to *Listeria monocytogenes* and *Escherichia coli* growth rates. It was presented in its most complete form in Ross et al. (2003):

$$\sqrt{\mu_{max}} = c \times (T - T_{min}) \times (1 - \exp(d \times (T - T_{max}))) \times \sqrt{a_w - a_{w\,min}} \times (1 - \exp(g \times (a_w - a_{w\max}))) \times \sqrt{1 - 10^{pH_{min} - pH}} \times \sqrt{1 - 10^{pH - pH_{max}}} \times \sqrt{1 - \frac{LAC}{U_{min} \times (1 + 10^{pH - pk_a})}} \times \sqrt{1 - \frac{LAC}{D_{min} \times (1 + 10^{pk_a - pH})}} \tag{3.10}$$

where *c*, *d,* and *g* are fitted parameters, *LAC* is the lactic acid concentration (m*M*), U_{min} the minimum concentration (m*M*) of undissociated lactic acid that prevents growth when all other factors are optimal, D_{min} the minimum concentration (m*M*) of dissociated lactic acid that prevents growth when all other factors are optimal, pK_a is the pH for which concentrations of undissociated and dissociated lactic acid are equal, reported to be 3.86 (Budavari, 1989), and all other terms are as previously defined.

One of the advantages of the square-root-type models, and the cardinal parameters models, is that their form enables them to be readily simplified into models for special cases; e.g., in Equation 3.10, if one factor is held constant then the terms involving that factor simply reduce to constants.

An example is a model developed for *Listeria monocytogenes* (Ross et al., in press; WHO/FAO, in press), in which the superoptimal water activity term is not relevant, and in which a term for the effect of nitrite on *L. monocytogenes* growth rate was also included. That novel term was based on analysis of the predictions of the Pathogen Modeling Program (Buchanan, 1993a; www.arserrc.gov/mfs/pathogen.htm). The fitted model is shown in Equation 3.11.

$$\sqrt{\mu_{max}} = 0.1626 \times (T - 0.60) \times (1 - \exp(0.129 \times (T - 51.0))) \times \sqrt{(a_w - 0.925)} \times \sqrt{1 - 10^{4.93 - pH}} \times \sqrt{1 - \frac{LAC}{4.55 \times (1 + 10^{\mathrm{pH} - 3.86})}} \times \sqrt{\left(\left(493 - NIT \times \left(1 + \frac{(6.5 - \mathrm{pH})}{2}\right)\right) \Big/ 493\right)} \tag{3.11}$$

where *NIT* is the concentration of nitrite and all other terms are as previously defined.

As shown in Figure 3.2, Equation 3.10 and Equation 3.11 represent a new generation of square-root-type models where the level of lactic acid in uences the

range of pH values for which growth is theoretically observed, reflecting the known interaction between pH and undissociated lactic acid, and also the individual growth rate suppressing effects of hydrogen ion concentration and undissociated lactic acid concentration. This was not the case for the environmental parameters included in Equation 3.1 to Equation 3.9. In those models, each term expressed how an environmental factor reduced the growth rate of a microorganism. However, for those models the expected multidimensional growth space was not influenced by levels of the different environmental parameters. This limitation of predictive models for growth rate has been recognized and has led, in part, to the development of growth/no growth models (discussed in Section 3.2.3 and Section 3.4). To make accurate predictions, a model can include terms to force the predicted growth rate to zero (Augustin and Carlier, 2000b; Le Marc et al., 2002). Alternatively, the probability of growth under the test conditions can first be assessed using a growth boundary model. If growth is possible, a growth rate model in combination with a lag time model can be used to estimate the extent of growth (Ross et al., in press; WHO/FAO, in press).

It is also notable that the pH and lactic acid terms in Equation 3.10 are effectively gamma-model type terms (see Section 3.2.2), in which the effect of the level of growth rate inhibitor is scaled between 0 and 1, where 1 represents no inhibition, i.e., the optimal level of that environmental factor. In the case of lactic acid, the optimal level would be 0, while for pH the optimum is ~7. This illustrates the close relationship between square-root-type models, and those that embody the gamma concept, such as the cardinal parameter models.

3.2.2 The Gamma Concept

The concept of dimensionless growth factors, now known as the gamma (γ) concept, was introduced in predictive microbiology by Zwietering et al. (1992). Later, minor changes and new developments were added (Wijtzes et al., 1998, 2001; Zwietering, 1999; Zwietering et al., 1996).

The gamma (γ) concept relies on:

1. The observation (e.g., Adams et al., 1991; McMeekin et al., 1987) that many factors that affect microbial growth rate act independently, and that the effect of each measurable factor on growth rate can be represented by a discrete term that is multiplied by terms for the effect of all other growth rate affecting factors, i.e.:

$$\mu = f(\text{temperature}) \times f(a_w) \times f(\text{pH}) \times f(\text{organic acid}) \\ \times f(\text{other}_1) \times f(\text{other}_2) \times \ldots . f(\text{other}_n)$$

2. That the effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate (i.e., the rate when that environmental factor is at the optimum level)

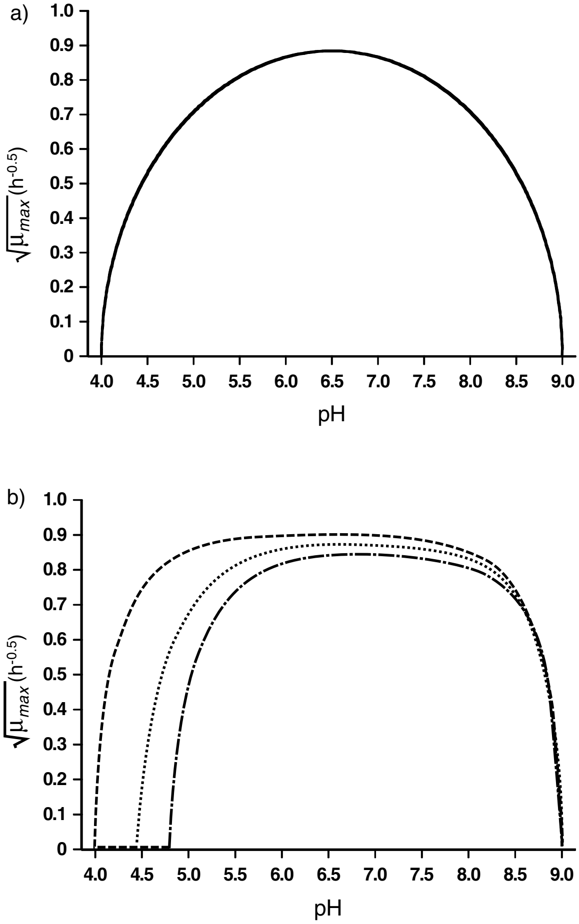


FIGURE 3.2 Simulation of Equation 3.7 (a) and Equation 3.10 (b) at a fixed temperature and water activity. pH_{min} is 4.0 and pH_{max} is 9.0. For Equation 3.10, U_{min} = 10 m*M* and D_{max} = 1000 m*M*. The concentrations of lactic acid (LAC) depicted are 0 m*M* (dashed line), 50 m*M* (dotted line), and 100 m*M* (dash-dotted line).

Under completely optimal conditions each microorganism has a reproducible maximum growth rate, notwithstanding the potential effect of strain variability. As any environmental factor becomes suboptimal the growth rate declines in a predictable manner, and the extent of that inhibition can be related to the optimum growth rate by calculating the relative rate at the test condition compared to that at the optimum. Thus, under the gamma concept approach, the cumulative effect of many factors poised at suboptimal levels can be estimated from the product of the relative inhibition of growth rate due to each factor, as indicated by Equation 3.12. The relative inhibitory effect of a specific environmental variable is described by a growth factor "gamma" (γ), a dimensionless measure that has a value between 0 and 1 (e.g., Equation 3.13 to Equation 3.15).

The relative inhibitory effect can be determined from the "distance" between the optimal level of the factor and the minimum (or maximum) level that completely inhibits growth by recourse to a predictive model. In the gamma model approach, the reference growth rate is μ_{max}, so that reference levels of temperature, water activity, etc. are those that are the optimum for growth rate, usually represented as T_{opt}, $a_{w\ opt}$, pH_{opt}, etc. The combined effect of several environmental factors is then determined by multiplication of their respective γ factors (Equation 3.16).

$$\gamma = \frac{\text{Growth rate at actual environmental conditions}}{\text{Growth rate at optimal environmental conditions}} = \frac{\mu_{\max}(T, a_w, \text{pH, etc.})}{\mu_{\max\ opt}} \tag{3.12}$$

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right)^2 \tag{3.13}$$

$$\gamma(a_w) = \frac{a_w - a_{w\ \min}}{1 - a_{w\ \min}} \tag{3.14}$$

$$\gamma(pH) = \frac{(pH - pH_{\min}) \cdot ((pH_{\max} - pH)}{(pH_{opt} - pH_{\min}) \cdot (pH_{\max} - pH_{opt})} \tag{3.15}$$

$$\mu_{\max} = \mu_{\max\ opt} \cdot \gamma(T) \cdot \gamma(a_w) \cdot \gamma(pH) \tag{3.16}$$

The effect of environmental parameters like carbon dioxide, sodium lactate, and nitrite has also been included in square-root-type models (see, e.g., Equation 3.8 to Equation 3.11). The absence of these inhibitory substances is optimal for growth and therefore the calculation of γ factors requires information only about the lowest concentration of each substance that prevents growth (or, similarly, the maximum level that can be tolerated before growth ceases) analogous to minimum inhibitory concentrations (MICs).

$$\gamma(CO_2) = \left(\frac{\%CO_{2\ \max} - \%CO_2}{\%CO_{2\ \max} - \%CO_{2\ opt}}\right)^2 = \left(\frac{\%CO_{2\ \max} - \%CO_2}{\%CO_{2\ \max}}\right)^2 \tag{3.17}$$

3.2.2.1 Expanding Existing Models

Given that there is a finite number of models (see Table 3.5 and Table 3.6), and that few models include factors of relevance to all foods, some workers have attempted to integrate terms for specific variables from one model into another to suit a specific

food and the conditions of interest. Because of the assumption of independent action of growth rate inhibitors, the dimensionless γ factors can, in principle, be readily exchanged between existing models and, at the time of writing, this is increasingly being done. Values of parameters like μ_{max}, μ_{opt}, T_{min}, T_{opt}, $a_{w\,min}$, pH_{min}, pH_{opt}, pH_{max}, and $\%CO_{2\,max}$ from which gamma factors can be derived are known for a considerable number of food-related pathogenic microorganisms. The approach was possibly taken to its logical conclusion by Augustin and Carlier (2000a,b) who collated, and integrated into a single model, literature data and observations for more than 15 factors in foods that affect the growth rate of *L. monocytogenes*.

For spoilage bacteria from chilled foods, growth kinetics at low temperatures are often well characterized but values of μ_{max}, μ_{opt}, T_{opt}, pH_{opt}, and pH_{max} are frequently unknown or have not been determined accurately. This is the case, for example, for the specific spoilage organisms *Photobacterium phosphoreum*, *Shewanella putrefaciens,* and *Brochothrix thermosphacta*. In this situation the classical gamma concept cannot be used to develop a secondary model. However, when a simple square-root-type model including the effect of temperature and, e.g., CO_2, has been developed for chilled product stored at a known pH (pH_{ref}) and water activity ($a_{w\,ref}$) then these models can be expanded at suboptimal growth conditions by addition of γ-like factors, as shown in Equation 3.18 (Dalgaard et al., 2003).

$$\begin{aligned}\sqrt{\mu_{max}} = \; & b \\ & \cdot\ (T - T_{min}) \\ & \cdot\ (\%CO_{2\,max} - \%CO_2) / \%CO_{2\,max} \\ & \cdot\ \sqrt{(a_w - a_{w\,min}) / (a_{w\,ref} - a_{w\,min})} \\ & \cdot\ \sqrt{(pH - pH_{min}) / (pH_{ref} - pH_{min})}\end{aligned} \tag{3.18}$$

Clearly, this approach should be used with some caution because the assumption of independent action has not been tested for all environmental factor combinations. Thus, the range of applicability of the expanded model should be evaluated, e.g., by comparison with data from challenge tests or naturally contaminated products (Giménez and Dalgaard, in press). (Section 3.2.5 discusses the expansion of existing polynomial models.)

3.2.3 Cardinal Parameter Models

Cardinal parameter models (CPMs) were introduced to predictive microbiology in 1993 and have become an important group of empirical secondary models (Augustin and Carlier, 2000a,b; Le Marc et al., 2002; Messens et al., 2002; Pouillot et al., 2003; Rosso, 1995, 1999; Rosso et al., 1993, 1995; Rosso and Robinson, 2001). The basic idea behind CPMs is to use model parameters that have a biological or graphical interpretation. When models are fitted to experimental data by nonlinear regression (see Chapter 4), this has the obvious advantage that appropriate starting values are easy to determine. General CPMs rely on the assumption that the inhibitory effect of environmental factors is multiplicative, an assumption that was

formalized in the gamma (γ) concept discussed above (Section 3.2.2). Thus, general CPMs consist of a discrete term for each environmental factor, with each term expressed as the growth rate *relative to that when that factor is optimal*; i.e., each term has a numerical value between 0 and 1. At optimal growth conditions all terms have a value of 1 and thus μ_{max} is equal to μ_{opt} (Equation 3.19).

Equation 3.19 to Equation 3.21 show a CPM that includes the effect of temperature (T), water activity (a_w), pH, inhibitory substances (c_i) and qualitative factors (k_j) on μ_{max} (Augustin and Carlier, 2000a). This extensive CPM was developed from available literature data from many studies for growth of *Listeria monocytogenes.* The inhibitory substances included (1) undissociated acetic acid, lactic acid, and citric acid, (2) Na-benzoate, K-sorbate, and the undissociated form of sodium nitrite, and (3) glycerol monolaurin, butylated hydroxyanisole, butylated hydroxytoluene, *tert*-butylhydroquinone, CO_2, caffeine, and phenol. In addition, the effect of competitive growth of microorganisms and the inhibitory effect due to specific types of foods were included in the model as qualitative factors.

$$\mu_{max} = \mu_{opt} \cdot CM_2(T) \cdot CM_2(a_w) \cdot CM_1(pH) \cdot \prod_{i=1}^{n} \gamma(c_i) \cdot \prod_{j=1}^{p} k_j \qquad (3.19)$$

$$CM_n = \qquad (3.20)$$

$$\begin{cases} 0, & X \le X_{min} \\ \dfrac{(X - X_{max}) \cdot (X - X_{min})^n}{(X_{opt} - X_{min})^{n-1} \cdot [(X_{opt} - X_{min}) \cdot (X - X_{opt}) - (X_{opt} - X_{max}) \cdot ((n-1) \cdot X_{opt} + X_{min} - n \cdot X)]}, & X_{min} < X < X_{max} \\ 0, & X \ge X_{max} \end{cases}$$

$$\gamma(c_i) = \begin{cases} (1 - c_i / MIC_i)^2, & c_i < MIC_i \\ 0, & c_i \ge MIC_i \end{cases} \qquad (3.21)$$

where X is temperature, water activity, or pH. X_{min} and X_{max} are, respectively, the values of X_i below and above which no growth occurs, X_{opt} is the value at which μ_{max} is equal to its optimal value μ_{opt}. MIC_i is the minimal inhibitory concentration of specific compounds above which no growth occurs.

Within predictive microbiology various CPMs were developed during the 1990s and in the same period different cardinal parameter temperature models were independently developed in other fields, e.g., to predict the effect of temperature on growth rates (r) of crops (Equation 3.22; Yan and Hunt, 1999; Yin and Wallace, 1995).

$$r = r_{max} \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right) \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right)^{\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}} \qquad (3.22)$$

TABLE 3.2
Parameter Values in Square-Root Type (Sqrt) and Cardinal Parameter Models (CPM)

Organism	T_{min}		T_{opt}		T_{max}		μ_{opt}		Reference
	Sqrt	CPM	Sqrt	CPM	Sqrt	CPM	Sqrt	CPM	
Escherichia coli	2.9	4.9	41.0	41.3	49.2	47.5	2.3	2.3	Rosso et al. (1993)
Salmonella Typhimurium	3.8	5.7	39.8	40.0	51.1	49.3	1.7	1.7	Oscar (2002)
	pH_{min}		pH_{opt}		pH_{max}		μ_{opt}		
	Sqrt	CPM	Sqrt	CPM	Sqrt	CPM	Sqrt	CPM	
Listeria monocytogenes	4.2	4.6	7.0	7.1	9.8	9.4	1.0	0.95	Rosso et al. (1995)

In several ways CPMs resemble square-root models and responses of the two types of models can be practically identical, e.g., for the effect of temperature, water activity, and pH (Oscar, 2002; Rosso et al., 1993, 1995). Parameters in the two types of models are typically named T_{min}, T_{max}, $a_{w\ min}$, $a_{w\ max}$, pH_{min}, and pH_{max}. However, these model parameters are not de ned in entirely the same way for CPMs and square-root-type models. In fact, when identical data are fitted to the two types of models square-root-type models estimate lower T_{min}, $a_{w\ min}$, and pH_{min} values and higher T_{max}, $a_{w\ max}$, and pH_{max} values (Table 3.2; see also Chapter 4).

T_{min} values estimated by CPMs and square-root-type models often differ by ~2°C as shown in Table 3.2. Table 3.3 shows that a 2°C difference of a T_{min} value has a pronounced effect on μ_{max} values predicted by both a square-root-type model and a CPM. Thus, parameter values estimated by using one of these types of models

TABLE 3.3
Effect of T_{min} Values (–1°C and +1°C) on μ_{max} Values Predicted by a Square-Root and a Cardinal Parameter Model at 4, 8, and 12°C

Temperature (°C)	Square-Root Model[a]			Cardinal Parameter Model[b]		
	μ_{max} (h^{-1})		% Difference	μ_{max} (h^{-1})		% Difference
	T_{min} = –1°C	T_{min} = +1°C		T_{min} = –1°C	T_{min} = +1°C	
4	0.0216	0.0078	64	0.0216	0.0087	60
8	0.0700	0.0424	40	0.0718	0.0485	33
12	0.1461	0.1046	28	0.1549	0.1237	20

[a] The model of Ratkowsky et al. (1983) used with values of the model parameters *b* and *c* selected to obtain a T_{opt} value ~37°C and a μ_{opt} value of ~ 1.0 h^{-1}. T_{max} was 45.0°C.
[b] The model of Rosso et al. (1993) used with T_{opt} of 37°C, μ_{opt} 1.0 h^{-1}, and T_{max} 45.0°C.

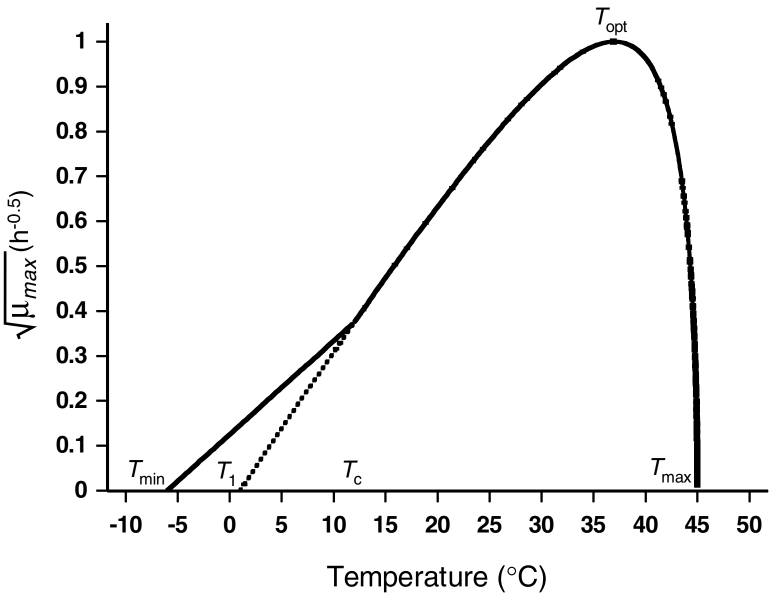


FIGURE 3.3 Simulation of the model $\mu_{max} = \mu_{opt} \times CM_2(T)$, with $CM_2(T)$ given by Equation 3.23 and with T_{min} of –6°C, T_1 of 1°C, T_c of 12°C, T_{opt} of 37°C, and T_{max} of 45°C. μ_{opt} was 1.0 h^{-1}.

cannot be used with the other type of model. This situation is similar to the estimation of μ_{max} values by some primary growth models. Modi ed Gompertz models (Gibson et al., 1987; Zwietering et al., 1990), e.g., overestimate μ_{max} by ~15% (Dalgaard et al., 1994; Membré et al., 1999; Whiting and Cygnarowicz-Provost, 1992) and their growth rate values should not be used together with the exponential, the logistic, or other Richards family of growth models that rely on accurate μ_{max} values.

Classical CPMs (Equation 3.19 and Equation 3.20) as well as square-root-type models describe a straight line relation between suboptimal temperatures and $\sqrt{\mu_{max}}$ (Figure 3.1 and Figure 4.4 [Chapter 4]). It has been reported by Bajard et al. (1996) that a different, biphasic, relationship can be observed for some strains of *Listeria monocytogenes*. More recently, Le Marc et al. (2002) observed a biphasic relationship for a strain of *Listeria innocua.* Le Marc et al. (2002) suggested an expanded CPM (Equation 3.23) to simulate this type of growth response (Figure 3.3). In Equation 3.23, T_c is the change temperature and T_1 corresponds to the T_{min} value in a classical cardinal temperature model (Rosso et al., 1993). McMeekin et al. (1993), however, cautioned against the interpretation of apparently continuously curved relationships as the combination of two linear responses, and provided a simple illustration of the effect. It should also be noted that other workers (e.g., Nichols et al., 2002a,b; see also Chapter 4) have not observed the "curvature" in the low temperature region of *L. monocytogenes* growth.

$$CM_2(T) = \begin{cases} \dfrac{(T-T_1)^2 \cdot (T-T_{\max})}{(T_{opt}-T_1)\cdot[(T_{opt}-T_1)\cdot(T-T_{opt})-(T_{opt}-T_{\max})\cdot(T_{opt}+T_1-2\cdot T)]} & , T_c < T < T_{\max} \\ \dfrac{(T_c-T_1)^2 \cdot (T-T_{\max})}{(T_{opt}-T_1)\cdot[(T_{opt}-T_1)\cdot(T_c-T_{opt})-(T_{opt}-T_{\max})\cdot(T_{opt}+T_1-2\cdot T_c)]} \cdot \left(\dfrac{T-T_{\min}}{T_c-T_{\min}}\right)^2 & , T_{\min} < T < T_c \end{cases} \tag{3.23}$$

As stated above, general CPMs rely on the assumption that different environmental parameters have independent and thereby multiplicative effects on μ_{max} (Equation 3.19). The successful use of many general CPMs and square-root-type models has shown this assumption to be reasonable for wide ranges of environmental conditions. However, numerous studies have shown that the growth range of a microorganism to one environmental condition is affected by other environmental factors (see Section 3.4). This suggests that the predictive accuracy of general CPMs can be improved by taking into account interactions between environmental parameters, particularly where one factor is sufficiently stringent that it reduces the growth range of the organism in other environmental "dimensions."

Various approaches have been suggested to describe growth limits under the influence of multiple variables (see Section 3.4.4). Two such approaches have been suggested for direct incorporation in CPMs and are discussed briefly here. Augustin and Carlier (2000b) developed a global secondary model for *L. monocytogenes,* including terms for interactions that prevented growth. Absolute minimal cardinal values $X^0_{\min}$ were estimated by assuming that all inhibitory substances were absent. Similarly, absolute minimal inhibitory concentrations MIC^0_i were estimated for optimal concentrations of other environmental parameters ($X = X_{opt}$). Then, interaction between environmental parameters was taken into account by modifying each of the $X^0_{\min}$ values (Equation 3.24) and the MIC^0_i values, depending on levels of other environmental parameters. After calculation of appropriate T_{min}, $a_{w\,min}$, pH_{min}, and MIC_i values, growth rates were then predicted by using Equation 3.19 to Equation 3.21.

$$X_{\min} = X_{opt} - (X_{opt} - X^0_{\min}) \cdot \left(\prod_{i=1}^{n} \left(1 - \frac{c_i}{MIC^0_i}\right) - \left(\frac{Y_{opt}-Y}{Y_{opt}-Y^0_{\min}}\right)^3 - \left(\frac{Z_{opt}-Z}{Z_{opt}-Z^0_{\min}}\right)^3 \right)^{1/3} \tag{3.24}$$

with *X*, *Y*, and *Z* being temperature, pH, or water activity.

A different approach was used by Le Marc et al. (2002) to model the interactive effects of temperature, pH, and concentration of undissociated organic acids (HA) on growth of *Listeria innocua.* Cardinal parameter values were kept constant and the space of environmental factors was divided into (1) the independent effect space ($\xi = 1$), (2) the interaction space ($0 < \xi < 1$), and (3) the no growth space ($\xi = 0$) (Equation 3.25 to Equation 3.27).

$$\mu_{\max} = \mu_{opt} \cdot CM_2(T) \cdot CM_1(pH) \cdot \tau([HA]) \cdot \xi(T, pH, [HA]) \tag{3.25}$$

$$\xi(\varphi(T, pH, ULA)) = \begin{cases} 1 & \psi \le \theta \\ 2(1-\psi) & \theta < \psi < 1 \\ 0 & \psi \ge 1 \end{cases} \tag{3.26}$$

$$\psi = \sum_i \frac{\varphi_{e_i}}{2\prod_{j \ne i}(1-\varphi_{e_j})} \tag{3.27}$$

with $\varphi_T = (1-\sqrt{CM_2(T)})^2$, $\varphi_{pH} = (1-CM_1(pH))^2$, and $\varphi_{Undissociated\ Lactic\ Acid\ (ULA)}$ = 1 – (ULA/MIC_{ULA})) and where e_i are the environmental factors. For calculation of $CM_2(T)$ and CM_1(pH), see Equation 3.20. Le Marc et al. (2002) selected a value of 0.5, which was used for θ.

The performance of the two approaches to model interaction between environmental parameters is considered in greater detail in Section 3.4.4. As shown above, CPMs that take into account the effect of interaction between environmental parameters are relatively complicated models. Thus, these models are not fully in agreement with the originally cardinal parameter modeling approach, i.e., that CPM uses only simple biological meaningful parameters that microbiologists are familiar with and that are easy to use by biologists (Rosso et al., 1993), and raises questions about whether those models are the most parsimonious forms available.

The model suggested by Augustin and Carlier (2000b) predicts the effect of interaction between temperature, pH, and lactic acid concentration on growth of *Listeria monocytogenes* to be more pronounced than the effect predicted for *Listeria innocua* by the model of Le Marc et al. (2002). For example, the Augustin and Carlier (2000b) model predicts no growth of *Listeria monocytogenes* at 8°C, pH 6.0, and with 200 m*M* of lactic acid, whereas at this condition the model of Le Marc et al. (2002) predicts growth and also that there is no interactive effect of the environmental factors (ξ = 1). Recently, Giménez and Dalgaard (in press) found the model of Augustin and Carlier (2000b) to substantially underestimate growth of *Listeria monocytogenes* in cold-smoked salmon. This could indicate that the model is in fact overestimating the importance of the interaction between at least some sets of environmental factors.

In a similar vein Ratkowsky and Ross (1995), recognizing the relationship between absolute limits for each environmental factor and their relationship to the parameters of square-root-type models and CPMs, experimented with the use of a kinetic model as the basis of a growth boundary model using linear logistic regression. This approach is discussed later (see Section 3.4.3.2).

The classical CPMs, in particular those including the effect of temperature, water activity, or pH, are now popular and used for many purposes within predictive microbiology (see Table 3.5 and Table 3.6). As one example a cardinal temperature and pH model has been combined with classical models of microbial kinetics, i.e.,

models that rely on yield factors and maintenance constants. In this way, production of curvacin A by *Lactobacillus curvatus* LTH 1174 growing in MRS broth was successfully modeled between 20 and 38°C and at pH values from 4.8 to 7.0 (Messens et al., 2002). Other examples include the use for CPMs to predict the radial growth rate of molds on solidi ed laboratory media (Panagou et al., 2003; Rosso and Robinson, 2001; Sautour et al., 2001). The ability of these models to predict growth in foods deserves further study.

For practical use of secondary predictive models it is important to know the precision of the predicted responses. With CPMs it has been suggested to determine cardinal parameters values for a number of different strains within each of the microbial species of interest (Membré et al., 2002). In this way a measure of intraspecies variability can be obtained. As an example, variability in the pH_{min} value for 10 strains of *E. coli* was ±0.20 corresponding to approximately four times the experimental error (Membré et al., 2002). More recently Pouillot et al. (2003) suggested the use of a CPM together with a Bayesian procedure for parameter estimation. This approach includes the use of hyperparameters and allows uncertainty (due to imperfect knowledge or data) and true variability (e.g., due to difference between strains) to be determined separately (see also Chapter 4). The approach seems most interesting and de nitely deserves to be studied further for different secondary predictive models.

3.2.3.1 Secondary Lag Time Models and the Concept of Relative Lag Time

When exponentially growing microorganisms are transferred from one environment into another, similar environment, growth usually continues without delay, i.e., a lag time is rarely observed. However, when the two environments differ, a lag time is often observed. Similarly, when microorganisms in the lag or stationary phases are transferred into identical or new environmental conditions a lag time may continue or result, respectively. Depending on the physiological state of the microorganisms, the magnitude of the shift in the environmental conditions, and the new environmental conditions themselves, the duration of the lag time may range from 0 to in nity.

Development of secondary lag time models is complicated by the fact that lag time is in uenced not only by the actual environmental conditions but also by previous environmental conditions and the physiological status of the cell, i.e., the growth phase of microorganisms at the time of transfer between environments and their “enzymatic readiness” to exploit the specific carbon and energy resources within the new environment. Within predictive microbiology, two main approaches have been used for development of secondary lag time models: (1) models where lag time and growth rate are modeled independently and (2) models where lag time is assumed proportional to the generation time. The latter group of models typically rely on the assumption that microorganisms need to perform a given amount of work to adapt to a new environment and that the rate at which this work can be done depends on the growth rate potential of the organism in the new environment (Robinson et al., 1998).

In the former approach, lag times or lag rates (i.e., reciprocal of lag time) are typically log-transformed to stabilize the variance of these data. Frequently, polynomial models (see Section 3.2.5) or artificial neural networks (see Section 3.2.6) have been used to develop independent secondary lag time models (Table 3.5). To model the effect of temperature downshifts, temperature upshifts, and physiological status of cells (e.g., exponential phase, stationary phase, starved, frozen, dried), separate polynomial models have been used for the different physiological conditions (Whiting and Bagi, 2002). When square-root-type and Arrhenius-type models are used for lag time modeling, lag *rates* are modeled or reciprocal forms of the growth rate models are used (see Section 3.2.1 and Section 3.2.4; Table 3.5 and Table 3.6).

Zwietering et al. (1994), e.g., used a square-root model (Equation 3.2) with identical values of the parameters T_{min}, c, and T_{max} to model lag time and growth rate — only the value of b differed between the two models. Specific secondary lag time models for particular environmental parameters have also been suggested, e.g., a hyperbola model for the effect of temperature (Equation 3.28; Oscar, 2002; Zwietering et al., 1994):

$$\lambda = \left[\frac{p}{T-q}\right]^m \tag{3.28}$$

where λ is the lag time, T the temperature, p the rate of change of lag time as a function of temperature, q the temperature at which lag time is in nite, and m is an exponent to be estimated.

Baranyi and Roberts (1994), Smith (1985), and McMeekin et al. (1993) have observed that lag times for identical inocula introduced to (at least some) environmental conditions are inversely proportional to growth rates and thus proportional to generation times (T_{gen}). This generalization has limits, however, as discussed further below and probably is most relevant to changes in environmental *temperature*. For example, Zwietering et al. (1994) showed that for the effect of temperature on *Lactobacillus plantarum* the product of μ_{max} and lag time (λ) was constant and had an average value close to 2. In these situations secondary lag time models can be derived directly from a growth rate model by using the simple concept of relative lag time (RLT; Equation 3.29) in common use but first defined by Mellefont and Ross (2003). Clearly, RLT reflects the physiological status of microorganisms introduced into a new environment as well as the difference between their actual and their previous environments, and can be interpreted as the amount of work the cell has to do to change its physiology (e.g., enzymes, membrane composition, number of ribosomes) to be able to grow at μ_{max} in that new environment.

Baranyi and Roberts (1994) suggested a primary model to estimate lag times from microbial growth curves and this model allowed determination of the parameters h_0, q_0, and α_0 all of which reflect the physiological state of microorganisms and, thereby, their readiness to grow in a given environment (Equation 3.29; Chapter 2). It can be seen that the parameter RLT is directly proportional to h_0.

$$\frac{\lambda}{T_{gen}} = RLT, \qquad \lambda = \frac{RLT \cdot \ln(2)}{\mu_{\max}},$$

$$\lambda \cdot \mu_{\max} = RLT \cdot \ln(2) = h_o = \ln\left(1 + \frac{1}{q_o}\right) = -\ln(\alpha_o) \tag{3.29}$$

where all parameters have meanings as indicated earlier.

Experimental methods to determine the physiological status of low levels of microorganisms in foods remain to be developed. Thus, for the time being these parameters have mainly theoretical importance.

The RLT concept is practically very useful for development of secondary lag time models, but it should be used with caution. Delignette-Muller (1998) evaluated data from nine studies where the effect of temperature, pH, NaCl, and $NaNO_2$ on lag time and generation time on different food-borne microorganisms had been modeled independently. In four of the nine studies, RLT was constant and an independent lag time model was not needed. However, primarily pH and NaCl in uenced RLT in the remaining studies. On the basis of large amounts of experimental data, Ross (1999a) showed the distribution of RLT of *B. stearothermophilus*, *Clostridium perfringens*, *E. coli*, *L. monocytogenes*, *Salmonella,* and *S. aureus* included peaks in the range 3 to 6 under a very wide range of experimental conditions. These distributions were similar to those presented by Augustin and Carlier (2000a), who observed a median RLT of 3.09 for *L. monocytogenes* (n = 1176). Using extreme environmental shifts, and severely growth-limiting outgrowth conditions, the hypothesis that RLT values have an upper limit was tested (Mellefont et al., 2003, in press). It was found that most RLTs were in the range 4 to 6, and that RLTs greater than 8 could not be induced within the experimental system employed. These observations suggest that while lag time is apparently highly variable, RLT is more uniform and reproducible. Distributions of RLT can be used in stochastic modeling studies, for example, microbial food safety risk assessments, where they could be used as plausible default assumptions if specific lag time information was not available. This approach can also simplify the growth modeling process because use of the RLT as a variable enables the effects of growth rate and lag to be predicted by a single growth rate model, as explained above.

The RLT concept implies that λ is at a minimum value ($\lambda_{\min}$) when the growth rate is optimal (μ_{opt}). This relation has been used together with CPMs to obtain simple secondary lag time models (Equation 3.30 and Equation 3.31; Augustin and Carlier, 2000a; Le Marc et al., 2002; Pouillot et al., 2003; Rosso, 1995, 1999a,b).

$$\lambda = \frac{\lambda_{\min} \cdot \mu_{opt}}{\mu_{\max}} \tag{3.30}$$

$$\lambda = \frac{\lambda_{\min}}{CM_2(T) \cdot CM_2(a_w) \cdot CM_1(\text{pH})} \tag{3.31}$$

For RLT models to be used in practice it must be known if, and to what extent, abrupt or smooth shifts in environmental parameters like temperature, pH, and water activity in uence RLT.

Data presented by Rosso (1999a,b) suggested that the effect of shifts in temperature and pH on growth of *E. coli* during fermentation of yoghurt was appropriately predicted by a CPM that relied on assumption of a constant RLT. Augustin et al. (2000) suggested a model to take into account the effect of growth phase and temperature history of *L. monocytogenes* on its RLT. For temperature downshifts the RLT increased from ~0 for a temperature shift of 0–5°C to ~2 for a downshift of 30–35°C. To model the effect of temperature downshifts and upshifts on RLT of *L. monocytogenes,* Delignette-Muller et al. (2003) recently used the data of Whiting and Bagi (2002) and suggested simple biphasic linear models. Separate models were used for inoculum with different physiological states. For *E. coli*, Mellefont and Ross (2003) found a similar effect of temperature downshifts whereas temperature upshifts had no systematic effect on RLT. For abrupt downshifts and upshifts in water activity the data of Mellefont et al. (2003) suggest that simple biphasic linear models, with different slopes for down- and upshifts, may be appropriate to predict RLT of both Gram-negative and Gram-positive food-borne bacteria. The universality of these responses remains unclear. For example, RLTs of *S. aureus* and *L. monocytogenes* were largely unaffected by abrupt osmotic shifts over a wide range of salt concentrations, whereas RLT of Gram-negative cells was strongly affected. More research is required before models that are as reliable as existing growth rate models can be developed for lag time, or RLT.

3.2.4 Secondary Models Based on the Arrhenius Equation

3.2.4.1 The Arrhenius Equation

The empirical Arrhenius–van't Hoff relationship:

$$\text{rate} = A\exp(\Delta E_a / RT) \tag{3.32}$$

or its mechanistic interpretation and modi cation due to Eyring (1935) based in absolute reaction-rate theory:

$$rate = KT\exp(\Delta H^{\ddagger} / RT) \tag{3.33}$$

where the parameters may be interpreted as follows: A is a constant related to the number of collisions between reactants per unit time, E_a the activation energy, R the gas constant (8.314 J/K/mol), T the temperature in Kelvin, K is similar to A but includes steric and entropic effects, and $\Delta H^{\ddagger}$ is the enthalpy difference between the

transition state complex and the reactants, are well established in chemistry to describe the effects of temperature on the rate of chemical reactions. Taking the logarithm of both sides of Equation 3.32:

$$\ln\,(rate) = \ln(A) \times \Delta E/RT$$

and reparameterizing the equation becomes:

$$\ln\,(rate) = \text{A'} + \left(\frac{\Delta E}{R}\right) \times \left(\frac{1}{T}\right)$$

Thus, if ln(rate) is plotted against $\left(\frac{1}{T}\right)$, the resulting plot is a straight line over temperature ranges relevant to microbial growth and allows estimation of the "activation energy" of the reaction, as shown in Figure 3.4. The activation energy can be used to characterize the reaction.

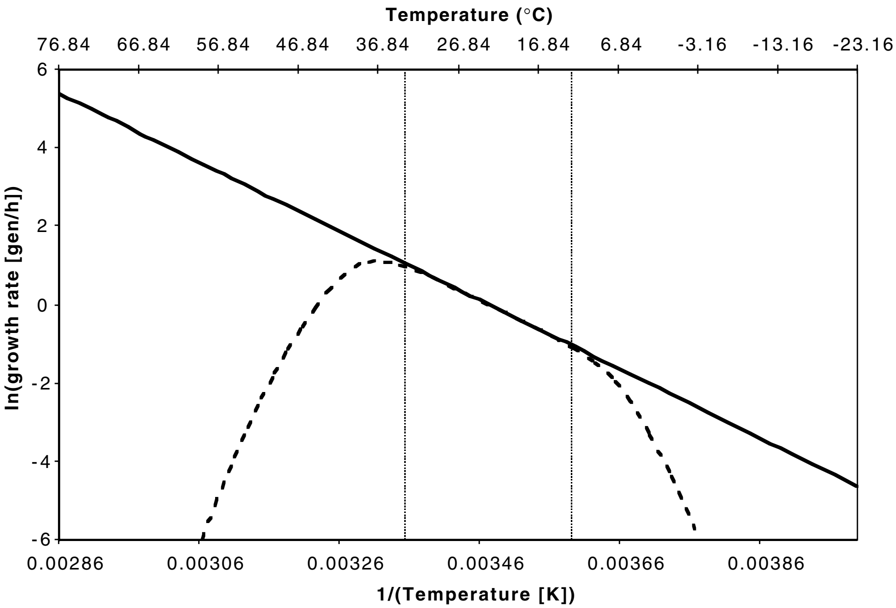


FIGURE 3.4 Diagram showing the effect of temperature on reaction rate predicted using the Arrhenius model (Equation 3.33; solid line) and the effect of temperature on microbial growth rate (dashed line) for a representative mesophilic organism. The "activation energy" is estimated from the slope of the solid line, multiplied by the universal gas constant. Over a narrow range of temperatures, the microbial growth rate follows the Arrhenius model prediction (Equation 3.29). This range has been termed the "normal physiological range" (NPR). At temperatures above or below the NPR, microbial growth rate deviates markedly from that predicted by the Arrhenius model.

It has been argued that because all life processes are the result of chemical reactions, the growth rate of organisms that cannot achieve thermal homeostasis should also be described by Arrhenius kinetics. Within a narrow range of temperature this is true. In practice, however, when microbial growth rate data for the full biokinetic temperature range are presented as an Arrhenius plot, the data are far from linear as shown in Figure 3.4, and confirmed by numerous studies (Heitzer et al., 1991; McMeekin et al., 1993; Schoolfield et al., 1981).

A range of secondary models, based on adherence to the reaction kinetics described by the Arrhenius model, but including terms to account for the observed deviations, have been proposed. These models fall into two groups:

1. Those based on putative mechanistic modifications of the Arrhenius models
2. Those based on empirical modifications

3.2.4.2 Mechanistic Modifications of the Arrhenius Model

Models in this category include those of Johnson and Lewin (1946) to describe the high-temperature growth of bacteria, Hultin (1955) to describe rates of enzymatic catalysis in the low temperature region, Sharpe and DeMichele (1977) who synthesized these two equations to produce a model for the temperature dependence of bacterial growth rate in the entire biokinetic region, the model of Schoolfield et al. (1981), which is a reparameterization of the Sharpe and DeMichele model to overcome difficulties in fitting by nonlinear regression, and the models of McMeekin et al. (1993) and Ross (1993a, 1999b). The latter models incorporate contemporary knowledge of the thermodynamics of protein folding to overcome failures in the Schoolfield et al. model related to unrealistic parameter estimate (Ratkowsky et al., 1991).

The above models were originally developed to provide an interpretation of microbial growth rates or enzyme-catalyzed reaction rates, in response to temperature but their mechanistic basis makes them attractive for use as secondary models.

This class of secondary models have previously been reviewed (McMeekin et al., 1993; Ratkowsky et al., 1991; Ross, 1999b; Ross and McMeekin, 1994). In summary, all of the models are based on the assumption that there is a single, enzyme-catalyzed, rate-limiting reaction in any microorganism. This reaction is characterized by an activation energy, which governs the rate of reaction in response to temperature, according to Arrhenius kinetics. Enzymes are proteins, however, and are themselves subject to the effects of temperature. The functional activity of enzymes is dependent upon their shape, or conformation, but they are flexible — the flexibility being required to achieve their catalytic function. Because temperature affects the bonds in the molecule, if the temperature changes too much, the conformation becomes so disrupted that denaturation takes place, both at high and low temperatures. These denaturation events are reversible, but at high temperatures if the temperature increases sufficiently, irreversible denaturation takes place (Ross, 1999b). Thus, these models include terms to model the probability, as a function of temperature, that the enzyme is in its metabolically active conformation and use this

estimate to modify the predictions of the Arrhenius model. Equation 3.34 to Equation 3.36 are examples of this form of model.

Model of Hinshelwood (1946):

$$\text{rate} = A_1 \exp\left(-E_a/RT\right) - A_2 \exp\left(-E_{a\,high}/RT\right) \tag{3.34}$$

where R, T, A, and E_a have the same meaning as above. $E_{a\,high}$ is the activation energy of the high-temperature denaturation of the rate-limiting enzyme.

Model of Schoolfield et al. (1981):

$$\frac{1}{K} = \frac{\rho_{(25)} \dfrac{T}{298} \exp\left\{\dfrac{H_A}{\mathrm{R}}\left(\dfrac{1}{298} - \dfrac{1}{T}\right)\right\}}{1 + \exp\left\{\dfrac{H_L}{\mathrm{R}}\left(\dfrac{1}{T_{1/2_L}} - \dfrac{1}{T}\right)\right\} + \exp\left\{\dfrac{H_H}{\mathrm{R}}\left(\dfrac{1}{T_{1/2_H}} - \dfrac{1}{T}\right)\right\}} \tag{3.35}$$

where T is the absolute temperature, R is the universal gas constant, and, for modeling bacterial growth, the other parameters have been interpreted as follows: K is the response (e.g., generation) time, $\rho_{(25)}$ a scaling factor equal to the response rate ($1/K$) at 25°C, H_A the activation energy of the rate-controlling reaction, H_L the activation energy of denaturation of the growth-rate-controlling enzyme at low temperatures, H_H the activation energy of denaturation of the growth-rate-controlling enzyme at high temperatures, $T_{1/2_L}$ the lower temperature at which half of the growth-rate-controlling enzyme is denatured, and $T_{1/2_H}$ is the higher temperature at which half of the growth-rate-controlling enzyme is denatured.

Model of Ross (1999b):

$$rate = \frac{CT \exp(\Delta H^{\ddagger} / RT)}{1 + \exp(-n(\Delta H^* - T\Delta S^* + \Delta C_p[(T - T^*_H) - T\ln(T/T^*_S)]) / RT)} \tag{3.36}$$

where C is a parameter whose value must be estimated, $\Delta H^{\ddagger}$ the activation enthalpy of the reaction catalyzed by the enzyme controlling the overall reaction rate, ΔC_p the difference in heat capacity (per mole amino acid residue) between the native (catalytically active) and denatured state of the enzyme, T_H* the temperature (K) at which the ΔC_p contribution to enthalpy is 0, T_S* the temperature (K) at which the ΔC_p contribution to entropy is 0, ΔH* the value of enthalpy at T_H* per mole amino acid residue, ΔS* the value of entropy at T_S* per mole amino acid residue, T the temperature (K), R the gas constant (8.314 J/K/mol), and n is the number of amino acid residues in the enzyme.

Equation 3.34 to Equation 3.36 include the simple Arrhenius model in the numerator of the equation. The denominators in Equation 3.35 and Equation 3.36, however, model the probability that the enzyme is in its active conformation. When

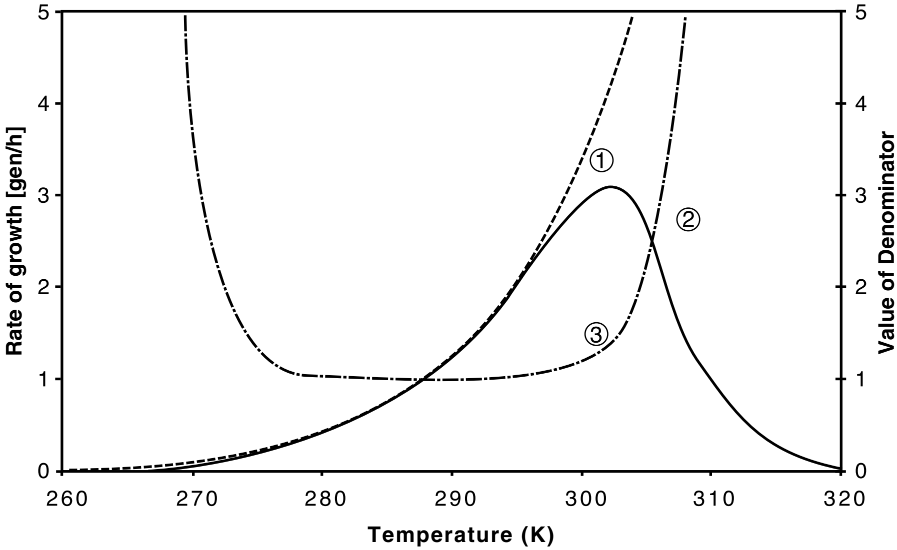


FIGURE 3.5 Diagram showing the interaction of terms in mechanistic models for microbial growth rate response to temperature. Curve 1 (dashed line) is the predicted growth rate in the absence of master enzyme denaturation, i.e., Arrhenius kinetics as modeled by the numerator of Equation 3.36. Curve 2 (dot-dash line) is the inverse of the probability of the "master enzyme" being in the active conformation, i.e., the denominator of Equation 3.36. Curve 3 (solid line) is the overall predicted rate from the model, i.e., the quotient of values in Curve 1 divided by values in Curve 2.

that probability is high, the denominator takes values close to 1, so that the overall rate is close to that predicted by the Arrhenius equation in the numerator. When the probability is lower, the value of the denominator increases, so that the observed rate is lower than that predicted by the numerator alone. These relationships are shown in Figure 3.5 for Equation 3.36, presented as rate vs. temperature for clarity of interpretation.

In practice, few of these types of models have been routinely applied in predictive microbiology, possibly because the models are highly nonlinear, and initial parameter estimates are dif cult to determine. Furthermore, it is currently not possible to independently measure the values of the parameters of the model because the putative master reaction has not been identified, and the concept that a single reaction is rate limiting under all environmental conditions seems improbable (Daughtry et al., 1997; Ross, 1999b). Finally, several workers (Heitzer et al., 1991; Ratkowsky, D.A., personal communication, 2003; Ross, 1993b) demonstrated that even with good quality data, square-root-type models provide an equally good fit as those "mechanistic" models, and are usually easier to work with. Examples of their use include Broughall et al. (1983) and Broughall and Brown (1984) who used the Schoolfield model, but also extended it to model the effect of water activity and pH, by replacing some terms in the model with polynomial expressions in a_w and pH. Adair et al. (1989) used a reparameterized form of the Schoolfield et al. model, which was

essentially another form of the Johnson and Lewin (1946) model. Recent studies (Ratkowsky et al., unpublished) have con rmed that Equation 3.36 does describe bacterial temperature–growth rate curves well for a wide range of species, and, in contrast with earlier models, that the estimated parameter values are realistic and consistent with the theoretical bases of the model.

3.2.4.3 Empirical Modifications of the Arrhenius Model

A second class of Arrhenius-based models for growth rate and reciprocal of lag time have been presented by Davey and coworkers. Davey (1989) introduced an Arrhenius-type model for the effects of temperature and water activity, which is linear and thus allows for explicit solution of the optimum parameter values. This model has the form:

$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2 \tag{3.37a}$$

where T is temperature (K), a_W has its usual meaning, and C_0, C_1, C_2, C_3, C_4 are coef cients to be determined.

Davey (1989) reported that the model described well seven data sets from the literature and subsequently demonstrated the ability of the model to also describe the reciprocal of lag phase duration (Davey, 1991). Davey (1994) fitted a variation of the model to the data of Adams et al. (1991) for *Yersinia enterocolitica* growth. The model included terms for temperature and pH, and is analogous to Equation 3.37a:

$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 pH + C_4 pH^2 \tag{3.37b}$$

where T is temperature (K), pH has its usual meaning, and C_0, C_1, C_2, C_3, C_4 are coef cients to be determined.

On the basis of these observations, Davey (1994) extended his earlier proposed general model structure for linear Arrhenius models (Davey, 1989) to account for the effect of multiple environmental factors affecting growth rate to the following form:

$$\ln(\text{rate}) = C_0 + \sum_{i=1}^{j} (C_{2i-1} V_i + C_{2i}^2 V_i) \tag{3.37c}$$

where j environmental factors, V, act in combination to affect the growth of the modeled organism, and C_0, C_1, C_2, …, C_j are coefficients to be determined.

This general form was applied by Davey and Daughtry (1995) to data of Gibson et al. (1988) for *Salmonella* growth in response to temperature, NaCl, and pH. Thus, their equation had the form:

$$\ln(rate) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3S + C_4S^2 + C_5pH + C_6pH^2 \qquad (3.37d)$$

where S is salt concentration (% w/v).

While the above model forms are empirical, they also recognize implicitly the temperature dependence of microbial growth rates. Daughtry et al. (1997) invoked chemical reaction rate theory to develop an alternative mechanistic model based on the Arrhenius equation. Those workers cited Levenspiel (1972) as stating that curvature in Arrhenius plots can arise if there are two, or more, reactions that "compete" to limit the reaction rate and dominate under different conditions so that the overall effect of temperature is the synthesis of the individual activation energies for the rate-limiting reactions at different temperatures. Daughtry et al. (1997) considered that bacterial growth was likely to be such a system.

By assuming that the "heat of reaction" (equivalent to the activation energy or activation enthalpy in the above discussion) is a polynomial function of temperature, the following modi ed Arrhenius model was developed:

$$\ln(rate) = C_0 + \frac{C_1}{T} + C_2 \ln T \qquad (3.38)$$

This model fitted experimental data as well as the temperature-only form of Equation 3.37a.

The "linear Arrhenius" or "Davey" models have been used to model growth of molds on solid microbiological media (Molina and Giannuzzi, 1999; Panagou et al., 2003). Panagou et al. (2003) preferred cardinal parameter and gamma-concept-type models (see Sections 3.2.2 and 3.2.3) over the Davey model because of their interpretable parameter values. Davey models have also been applied to UV and thermal inactivation and data describing the combined effects of pH and water activity on thermal inactivation, including vitamin denaturation (see Section 3.3), but they have not been widely adopted by other workers. McMeekin et al. (1993) and Davey (2001) identi ed a close correlation between estimates of coef cients C_1 and C_2, and C_3 and C_4, of Equation 3.37a, suggesting that the model was overparameterized.

3.2.4.4 Application of the Simple Arrhenius Model

For the entire biokinetic temperature range, growth rates of microorganisms are described less appropriately by the Arrhenius-type equations (Equation 3.34 to Equation 3.36) than by square-root-type and cardinal parameter models (see Section 3.2.4.2; Rosso et al., 1993; Zwietering et al., 1991). However, Arrhenius-type models remain useful as secondary kinetic models for less extensive ranges of storage temperatures (Table 3.5 and Table 3.6). Koutsoumanis and Nychas (2000) used Equation 3.32 to model the effect of temperatures between 0 and 15°C on μ_{max} and reciprocal lag time of naturally occurring pseudomonads growing aerobically on a type of Mediterranean sh. Koutsoumanis et al. (2000) also expanded the classical

Arrhenius model to take into account the combined effect of temperature and CO_2 on growth rates of spoilage bacteria in modified atmosphere packed fresh fish (Equation 3.39).

$$\ln(\mu_{\max}) = \frac{E_a}{R} \times \left(\frac{1}{T_{ref}} - \frac{1}{T} \right) + \ln(\mu_{ref} - d_{CO_2} \times \% CO_2) \qquad (3.39)$$

where T, E_a, and R have their usual meaning, $\%CO_2$ is the equilibrium concentration of CO_2 in the headspace gas, d_{CO_2} is a constant expressing the effect of CO_2 on μ_{max} and T_{ref} and μ_{ref} are temperature and maximum specific growth rate, respectively, at 273 K and 0 $\%CO_2$. The term including d_{CO_2} in Equation 3.39, describing CO_2 inhibition of growth rate, was previously suggested by Kalina (1993).

The simple Arrhenius model has also been used to calculate relative rates of spoilage (RRS) (Equation 3.37). RRS for a food product is de ned as the shelf life (determined by sensory evaluation) at a reference temperature (T_{ref}) divided by the shelf life observed at the actual storage temperature (Equation 3.40).

$$RRS = \frac{\text{Shelf life at } T_{ref}}{\text{Shelf life at } T} = \exp\left[\frac{E_A}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \qquad (3.40)$$

where T, E_a, and R have their usual meaning and T_{ref} is a reference temperature at which the shelf life is known.

RRS models are interesting because they enable shelf life to be predicted at different temperatures and for products where the specific spoilage organisms or the type of reaction responsible for spoilage are not known. For an unusually temperature-sensitive modified atmosphere packed shrimp product (E_a > 100 kJ/mol), Equation 3.40 described the effect of temperature (0 to 25°C) on shelf life more appropriately than a similarly formulated RRS model relying on the square-root model (Equation 3.1). However, a simple exponential RRS model was as useful as Equation 3.40. That the Arrhenius and exponential RRS models performed better than the square-root model was due to the fact that different groups of microorganisms were responsible for spoilage at low and high storage temperatures, respectively (Dalgaard and Jørgensen, 2000). This situation is common and a reason why entirely empirical RRS models can be more appropriate for shelf-life prediction than kinetic models relying on growth of known spoilage microorganisms. In fact, kinetic models for growth of spoilage bacteria are generally useful only for shelf-life prediction within the spoilage domain of a specific microorganism (Dalgaard, 2002).

3.2.5 Polynomial and Constrained Linear Polynomial Models

Of the types of secondary models applied within predictive microbiology polynomial models are probably the most common. As shown in Table 3.5 and Table 3.6,

the effect of many different environmental parameters (e.g., temperature, NaCl/a_w, pH, nitrite, CO_2, organic acids, and natural antimicrobials) has been described by these linear models. Polynomial models were extensively used during the 1990s and they remain widely applied although square-root-type and CPMs are becoming increasingly popular (Table 3.5). Polynomial models are attractive, rst, because they are relatively easy to fit to experimental data by multiple linear regression, which is available in most statistical packages. Second, polynomial models allow virtually any of the environmental parameters and their interactions to be taken into account. Thus, application of polynomial models is a simple way to summarize information from a data set. Once the coefficients in a polynomial model have been estimated, the information is easy to use particularly if the model is included in application software. In fact, the application software packages Pathogen Modeling Program and Food MicroModel rely primarily on the use of polynomial models (www.arserrc.gov/mfs/pathogen.htm; Buchanan, 1993a; McClure et al., 1994a).

To illustrate the use of polynomial models a quadratic equation used by McClure et al. (1993) is shown below (Equation 3.41):

$$\begin{aligned} \ln y = p_1 + p_2 x_1 + p_3 x_2 + p_4 x_3 + p_4 x_1 x_2 + p_6 x_1 x_3 \\ + p_7 x_2 x_3 + p_8 x_1^2 + p_9 x_2^2 + p_{10} x_3^2 + e \end{aligned} \quad (3.41)$$

where $\ln y$ denotes the natural logarithm of the modeled growth responses ($y = \mu_{max}$, lag time or maximum population density [MPD], or the modified Gompertz model parameters *B* or *M*); p_i ($i = 1, \ldots, 10$) are the coefficients to be estimated; x_1 is the temperature (°C); x_2 is the pH; x_3 is NaCl (% w/v); *e* is a random error supposed to have zero mean and constant variance.

As shown by Equation 3.41 the same polynomial equation can be used to model different microbial growth responses. Actually, many studies have modeled the effect of environmental conditions on specific parameters in primary growth models, particularly *B*, *M,* and *C* in the modified Gompertz model. Measures of lag time, growth rate, or time for, e.g., a 1000-fold increase in the cell concentration are then calculated at specific environmental conditions from the predicted value of *B*, *M,* and *C* (Buchanan and Phillips, 2000; Eifert et al., 1997; Skinner et al., 1994; Zaika et al., 1998). Growth responses to be modeled are typically ln- or $\log_{10}$-transformed (Equation 3.41) and it is common practice to transform the growth response without transforming the model.

However, polynomial models have properties that limit their usefulness as secondary predictive models. Polynomials include many coefficients that have no biological interpretation. As an example, Equation 3.41 uses 10 coefficients to model the effect of three environmental parameters. With four environmental parameters, polynomials with 15 coefficients are frequently used. The high number of coefficients and their lack of biological interpretation make it dif cult to compare polynomial models with other secondary predictive models. The important information included in, e.g., the T_{min} parameter of a square-root-type model, is not provided by a polynomial model.

Higher order polynomial models, e.g., cubic or quadratic models have been criticized for being too flexible and for attempting to model, rather than eliminate, experimental error (Chapter 4; Baranyi et al., 1996; Sutherland et al., 1996). Because of the very flexible nature of higher order polynomial models they should not be used as secondary models within predictive microbiology unless very high quality experimental data are available and support the application of these models. Furthermore, because quadratic polynomial models are highly flexible they should only be used to provide predictions by interpolation. Baranyi et al. (1996) pointed out that the interpolation region of a polynomial model is the minimum convex polyhedron (MCP) defined by the ranges of the environmental parameters used to develop the model, i.e., the experimental design. These authors also stressed that the interpolation region (Figure 3.10) can be substantially smaller than the rectangular parallelepiped whose sides are given by the endpoints of the ranges of environmental parameters, termed the "nominal variable space" (Baranyi et al., 1996).

Determination of the interpolation region of a polynomial model is not self-evident and requires information about ranges of the environmental parameters used to develop the model. Pin et al. (2000) suggested a method to determine if a specific environmental condition is inside or outside the interpolation region of a particular polynomial model. This method relies on the iterative algorithm used by the Solver add-in of Microsoft Excel and thus is readily accessible to many users. However, we believe for it to become widely used the calculation of interpolation regions should be included in dedicated predictive modeling application software.

To overcome the problem that quadratic polynomial models can be too exible, and therefore in some situations provide predictions that are not logical, the application of constrained polynomial models was recently suggested (Geeraerd et al., in press). With this approach, the basic idea is to combine *a priori* information about the effect of environmental parameters on growth responses with classical polynomial models. For example, at suboptimal conditions it was assumed that the growth rate should always increase for increasing temperature and a_w values and decrease for increasing CO_2 levels. Thus, the partial derivative of the model with respect to temperature and a_w should always be positive whereas the partial derivative of the model with respect to CO_2 should always be negative. Coef cients of the polynomial model were then fitted with the constraints obeyed at all edges of the experimental design. The constrained polynomial model was fitted by the usual process of minimizing the sum of squared errors and the tting was carried out using the Optimization Toolbox within the MatLab software (Geeraerd et al., in press). As compared to classical polynomial models, constrained polynomial models have the clear advantage of being more robust but the clear disadvantage of being substantially more dif cult to t. Simpli cation of the tting process seems necessary before constrained polynomial models nd wide application in predictive microbiology.

Masana and Baranyi (2000a) described methods for integration of new data into existing polynomial models, pointing out that the interpolation region of the newly developed model can be unexpectedly small and also presenting methods for quantifying the increased risk of inadvertent extrapolation (Baranyi et al., 1996). Polynomial models feature many cross-product terms, making the addition of new terms much more complex than with models embodying the gamma concept (Section

3.2.2). Nonetheless, when expanding a model by the addition of data for a new variable, Masana and Baranyi (2000b) demonstrated that the original model can be retained as a special case of the expanded model, by holding the terms of the original model, i.e., those that do not contain the new variable, as constants during the model fitting process for the expanded model.

3.2.6 Artificial Neural Networks

Artificial neural networks (ANNs) are algorithms that can be used to perform complex statistical modeling between a set of predictor variables and response variables. Their particular advantage is that they have the potential to approximate underlying relationships of any complexity between those variables. They have been used to generate secondary models for microbial growth rates and lag times (Garcia-Gimeno et al., 2002, 2003; Geeraerd et al., 1998a; Jeyamkondan et al., 2001; Lou and Nakai, 2001; Najjar et al., 1997), growth under fluctuating environmental conditions (Cheroutre-Vialette and Lebert, 2000; Geeraerd et al., 1998a), microbial inactivation (Geeraerd et al., 1998b), and have been proposed as an alternative to logistic regression modeling techniques (Tu, 1996). Their potential to replace logistic regression for growth limits modeling (see Section 3.4) has also been described (Hajmeer and Basheer, 2002, 2003a,b) in which context they have been termed "probabilistic neural networks" (PNNs).

Hajmeer et al. (1997) and Hajmeer and Basheer (2002, 2003a) describe the principles of ANNs and related technologies in the context of predictive microbiology, and numerous texts are dedicated to the subject but the following is largely drawn from the succinct and lucid description of Tu (1996).

Artificial neural networks were conceived decades ago by researchers attempting to reproduce the function of the human brain, i.e., its ability to learn and remember, but it was only in the 1980s that the "back-propagation" technique was rediscovered, enabling such computational systems to "learn" mathematical relationships between input and output variables.

Neural networks are effectively a series of mathematical relationships between predictor variables ("input nodes"), a series of hidden "nodes," and an output variable ("output node") (Figure 3.6). Each input node is related to each hidden node, and each hidden node is related to the output node, by some mathematical function. Each input is given a weight during the "training" routines, the value of each hidden node being the sum of a weighted linear combination of the input node values. In addition, bias values can be added to the weighted values of the inputs. These are analogous to the intercept in regression equations, while the weights are analogous to coefficients of the independent variables. The output node receives a weighted input from each of the hidden nodes, to which is often applied a logistic transformation or other function (the "activation function") to determine the overall output.

A set of input and corresponding output values is presented to the network, the error is evaluated, and the weights are then adjusted to minimize the difference between the predicted output and that which was observed. This process of adjustment of weights is the back-propagation step and involves algorithms based on complex equations. Input data are continuously presented to the neural network until

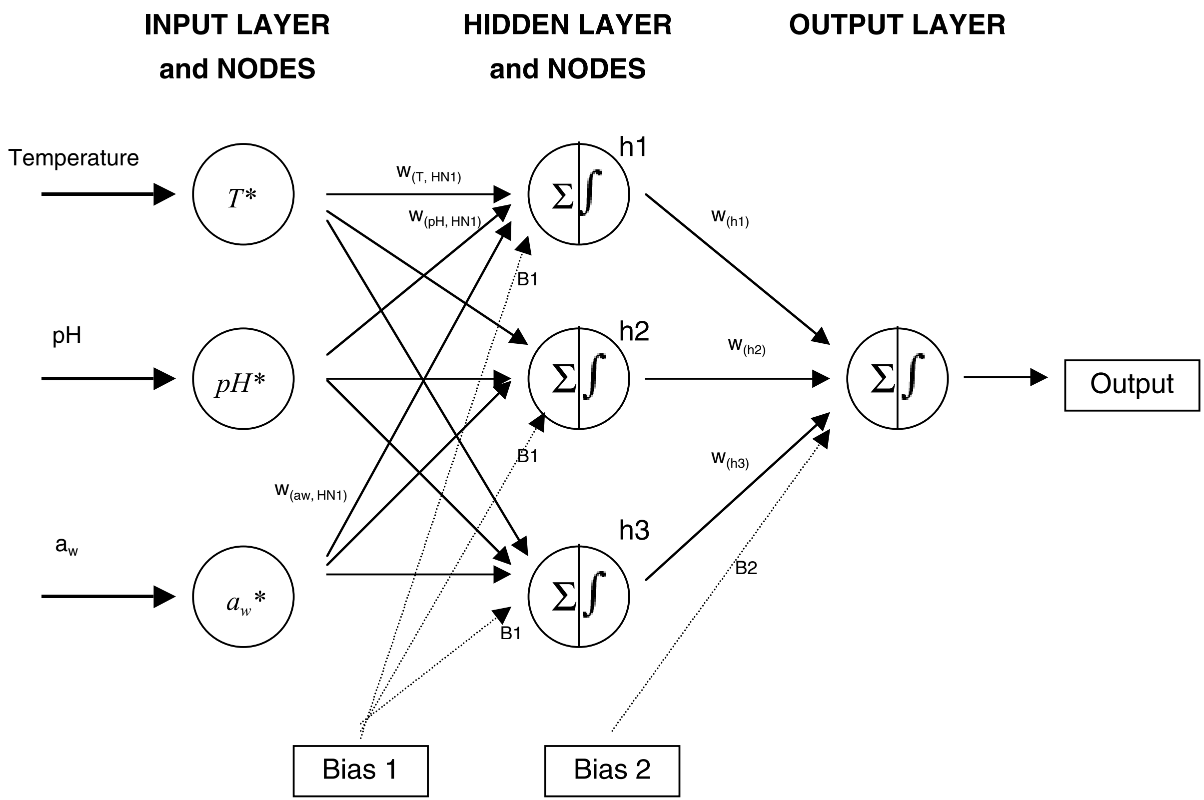


FIGURE 3.6 Diagram of an imaginary arti cial neural network that might be used in predictive microbiology. The output is the response of the population of microorganisms to variations in the temperature, pH, and water activity of their growth medium. (The diagram is fully explained in the text.)

the overall error has been minimized, a process analogous to the iterative routines employed in nonlinear regression software. Optimal training algorithms can, at this time, only be determined empirically. Additionally, when using ANNs other elements of the modeling require experimentation, including the number of training cycles (too many can reduce predictive performance), the number of nodes in the hidden layer, and the ideal learning rate (the magnitude of change in the weights for each training case).

In Figure 3.6, the input, hidden, and output layers are shown, as well as the connections between them. Nodes are represented by circles. The $W_{(i,j)}$ terms indicate the weight applied to the inputs to hidden nodes. (Not all weights are represented in the diagram.) The hidden nodes have a transformation applied to them, e.g., a logistic function represented by the functions $h1$, $h2$, etc. Thus, in the example:

$$\mathrm{h1} = 1/(1+\exp(\mathrm{Bias\ 1} + \mathrm{W}_{(\mathrm{T,\ HN1})} \times T^* + \mathrm{W}_{(\mathrm{pH,\ HN1})} \times pH^* + \mathrm{W}_{(\mathrm{aw,\ HN1})} \times a_w^*))$$

and, similarly:

$$\mathrm{Output} = 1/(1+ \exp(\mathrm{B2} + \mathrm{W}_{(\mathrm{h1})} + \mathrm{W}_{(\mathrm{h2})}))$$

Sufficient data are required so that a subset of data can be used to train the ANN, while the remainder is used to test the predictive ability of the ANN. One complete cycle of the training data set is called an "epoch" and the duration of the training is often described as the number of epochs required to minimize the error in the training set.

Tu (1996) compared the advantages and disadvantages of the ANN approach to those of traditional statistical regression modeling, as summarized in Table 3.4. Evaluation of the approach as applied to predictive models for microbial growth is presented below, and in relation to growth limits models in Section 3.4.2. Further comment is provided in Chapter 4, Section 4.4.3.

The use of ANN in predictive growth modeling remains relatively little developed, and direct comparison of the performance of different ANN techniques is still lacking. To describe growth curves, Schepers et al. (2000) concluded ANN was less appropriate than classical nonlinear sigmoidal growth models. Cheroutre-Vialette and Lebert (2000), however, found a recurrent (i.e., back-propagation) ANN suitable to predict growth of *Listeria monocytogenes* under constant and fluctuating pH and NaCl conditions. As shown in Table 3.5 and Table 3.6, several secondary ANN models have been developed including models for *Aeromonas hydrophila*, *Brochothrix thermosphaca*, *Escherichia coli*, lactic acid bacteria, *Listeria monocytogenes,* and *Shigella flexneri*. These secondary ANN models have been compared with polynomial, square-root-type, and cardinal parameter models. The comparisons showed ANN typically fitted experimental data better and in most cases provided slightly more accurate predictions. Thus, in general, ANN may provide slightly improved predictions. Commercial neural network software is available and development of ANNs has become relatively easy. However, ANN is a data-driven approach and this could be a drawback because a secondary model that can be written as an equation with coefficients and parameters is not produced. The

TABLE 3.4
Advantages and Disadvantages of Neural Network Approaches to Modeling

Disadvantages	Advantages
Neural networks are a "black box" and have limited ability to specifically identify possible causal relationships between predictor and response variables	Neural networks require less formal statistical training to develop
Neural network models may be more difficult to use in the field	Neural network models can implicitly detect complex nonlinear relationships between predictor and response variables
Neural network modeling requires greater computational resources	Neural network models have the ability to detect all possible interactions between predictor variables
Neural network models are prone to overfitting	Neural networks can be developed using multiple different training algorithms
	Neural network model development is empirical, and many methodological issues remain to be resolved

Source: After Tu, J.V. *J. Clin. Epidemiol.*, 11, 1225–1231, 1996.

incorporation of classical secondary models in user-friendly application software has been essential for the usefulness of predictive microbiology in industry, teaching, and research. It remains to be demonstrated whether successful ANN models can, in a similar way, be communicated to and conveniently applied by wide groups of users within predictive microbiology.

3.3 SECONDARY MODELS FOR INACTIVATION

There are relatively few models that consider the effects of multiple environmental factors on the rate of death of microorganisms, and these are discussed in Chapter 2 and Chapter 5. Some available inactivation models are also summarized in Table 3.5 and Table 3.6.

3.4 PROBABILITY MODELS

3.4.1 Introduction

Models to predict the likelihood, as a function of intrinsic and extrinsic factors, that growth of a microorganism of concern could occur in a food were first explored in the 1970s. Those models were concerned with prediction of the probability of formation of staphylococcal enterotoxin or botulinum toxin within a specified period of time under defined conditions of storage and product composition (Genigeorgis, 1981; Gibson et al., 1987). Phenomena that have been modeled using this approach

TABLE 3.5
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

Microorganisms and References	Type of Secondary Model	Response Variables	Independent Variables and Ranges
Aeromonas hydrophila			
Palumbo et al. (1992)[a]	Polynomial	GT,[b] lag	*T* (5–42°C); NaCl (0.5–4.5%); pH (5.0–7.3); Na-nitrite (0–200 ppm); anaerobic
McClure et al. (1994b)	Polynomial	GT, lag	*T* (3–20°C); NaCl (0.5–4.5%); pH (4.6–7.0); aerobic
Palumbo et al. (1996)[a]	Polynomial	GT, lag	*T* (5–42°C); NaCl (0.5–4.5%); pH (5.0–7.3); Na-nitrite (0–200 ppm); aerobic
Devlieghere et al. (2000a)	Square-root, polynomial	μ_{max}, lag	*T* (4–12°C); a_w (0.974–0.992); CO_2 (0–2403 ppm); pH 6.12; nitrite (22 ppm)
Jeyamkondan et al. (2001)	ANN	GT, lag	Data from McClure et al (1994b)
Aspergillus spp.			
Pitt (1995)	Kinetic with yield factors	Growth and a atoxin formation	*T*; a_w; pH; and colony size: limits not specified in manuscript
Molina and Giannuzzi (1999)	Arrhenius	Colony growth	*T* (25–36°C); propionic acid (129–516 ppm)
Rosso and Robinson (2001)	CPM	Colony growth	*T* (25, 30, 37°C); a_w (0.83–0.99); pH (6.5); humectant: glucose/fructose
Sautour et al. (2001)	CPM	Colony growth	*T* (25°C); a_w (0.88–0.99)
Bacillus cereus			
Benedict et al. (1993)[a]	Polynomial	GT, lag	*T* (5–42°C); NaCl (0.5–5.0%); pH (4.5–7.5); Na-nitrite (0–200 ppm); aerobic
Sutherland et al. (1996)[c]	Polynomial	GT, lag	*T* (10–30°C); NaCl (0.5–10.5%); pH (4.5–7.0); CO_2 (10–80%)
Zwietering et al. (1996)	Gamma	μ_{max}	*T* (10–30°C); a_w (0.95–1.00); pH (4.9–6.6)
Chorin et al. (1997)	Polynomial	Growth rate, lag	*T* (7–30°C); a_w (0.95–0.991); pH (5–7.5); humectant: glycerol
Singaglia et al. (2002)	Polynomial	Spore germination	*T* (20–40°C); a_w (0.94–0.99); pH (4.5–6.5)
Clostridium botulinum			
Baker and Genigeorgis (1990)[a]	Polynomial	Time to toxin formation	*T* (4–30°C); initial spore conc. (–2 to +4 log cfu/g); initial aerobic plate count (–2 to +3 log cfu/g)
Graham et al. (1996)[c]	Polynomial	GT, time to toxin	*T* (4–30°C); NaCl (1.0–5.0%); pH (5.0–7.3)

TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

Microorganisms and References	Type of Secondary Model	Response Variables	Independent Variables and Ranges
Whiting and Oriente (1997)[a]	Polynomial	Time to turbidity	*T* (4–28°C); NaCl (0–4%); pH (5–7); initial spore conc. (1–5 log cfu/g)
Chea et al. (2000)	Polynomial	Spore germination	*T* (15–30°C); NaCl (0.5–4.0%); pH (5.5–6.5)
Fernandez et al. (2001)	Polynomial	Time to turbidity	*T* (5–12°C); NaCl (0.5–2.5%); pH (5.5, 6.5); CO_2 (0–90%)
Clostridium perfringens			
Juneja et al. (1996)[a]	Polynomial	GT, lag	*T* (12–42°C); NaCl (0–3%); pH (5.5–7.0.); Na-pyrophosphate (0–3%)
Escherichia coli			
Buchanan and Bagi (1994)[a]	Polynomial	GT, lag	*T* (5–42°C); NaCl (0.5–5.0%); pH (4.5–8.5); Na-nitrite (0–200 ppm); aerobic and anaerobic
Sutherland et al. (1995, 1997)[c]	Polynomial	GT, lag	*T* (10–30°C); NaCl (0.5–6.5%); pH (4.0–7.0); Na-nitrite (0–200 ppm); aerobic
Rasch (2002)	Polynomial	Growth rate	*T* (10–30°C); NaCl (0.5–3.0%); pH (4.5–6.5); reuterin (0–4 AU/ml)
Ross et al. (2003)	Square-root	GT	*T* (7.6–47.4°C); a_w (0.951–0.999); pH (4.02–8.28); lactic acid (0–500 m*M*)
Skandamins et al. (2002)	Vitalistic	Time to decline in cell concentration	*T* (0–15°C); pH (4.0–5.0); oregano essential oil (0.0–2.1%)
Garcia-Gimeno et al. (2003)	ANN	Growth rate, lag time	*T* (9–21°C); NaCl (0–8%); pH (4.5–8.5); Na-nitrite (0–200 ppm)
Whiting and Golden (2003)	Polynomial	Time to decline in cell concentration	*T* (4–37°C); NaCl (0–15%); pH (3.5–7.0); Na-lactate (0–2%); Na-nitrite (0–75 ppm)
Listeria monocytogenes[d]			
Buchanan et al. (1997)	Polynomial	Time to decline in cell concentration	*T* (4–42°C); NaCl (0.5–19%); pH (3.2–7.3); lactic acid (0–2%); Na-nitrite (0–200 ppm)
Razavilar and Genigeorgis (1998)	Polynomial	Probability of growth	*T* (4–30°C); NaCl (0.5–12.5%); methyl paraben (0–0.2%); pH (~5.9); K-sorbate (0.3%); Na-propionate (0.1%); Na-benzoate (0.1%)
Cheroutre-Vialette and Lebert (2000)	ANN	Absorbance at 600 nm	*T* (20°C); pH (5.6–9.5); NaCl (0–8%)

TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

Microorganisms and References	Type of Secondary Model	Response Variables	Independent Variables and Ranges
Bouttefroy et al. (2000)	Polynomial	Cell concentration	*T* (22°C); pH (5.0–8.2); NaCl (0–6%); curvaticin 13 (0–160 AU/ml)
Rodriguez et al. (2000)	Arrhenius	μ_{max}	*T* (4–20°C)
Augustin and Carlier (2000a,b)	CPM	μ_{max}, lag	*T* (−2.7 to −45.5°C); a_w (0.910–0.997); pH (4.55–9.61); acetic acid (0–20.1 m*M*); lactic acid (0–5.4 m*M*); citric acid (0–1.6 m*M*); Na-benzoate (0–0.7 m*M*); K-sorbate (0–5.1 m*M*); Na-nitrite (0–11.4 μ*M*); glycerol monolaurin (0–118.5 ppm); butylated hydroxyanisole (0–254 ppm); butylated hydroxytoluene (0–48.7 ppm); *tert*-butylhydroquinone (0–1400 ppm); CO_2 (0–1.64 proportion); caffeine (0–10.8 g/l); phenol (0–12.5 ppm)
Ross et al. (in press)	Square-root	μ_{max}	*T* (3–40 °C); a_w (0.920–0.997); pH (4.0–7.8); lactic acid (0–450 m*M*); nitrite (0–150 ppm)
Buchanan and Phillips (2000)	Polynomial	GT, lag	*T* (4–37°C); pH (4.5–7.5); NaCl (0.5–10.5%); Na-nitrite (0–1000 ppm); aerobic
Buchanan and Phillips (2000)	Polynomial	GT, lag	*T* (4–37°C); pH (4.5–8.0); NaCl (0.5–5.0%); Na-nitrite (0–1000 ppm); anaerobic
Devlieghere et al. (2001)	Square-root, polynomial	μ_{max}, lag	*T* (4–12°C); a_w (0.9622–0.9883); Na-lactate (0–3.0%); Na-nitrite (20 ppm); pH (6.2)
Le Marc et al. (2002)	CPM	μ_{max}, lag	*T* (0.5–43°C); pH (4.5–9.4); acetic acid (16–64 m*M*); lactic acid (40–138 m*M*); propionic acid (18–55 m*M*)
Seman et al. (2002)	Polynomial	Growth rate	*T* (4°C); NaCl (0.8–3.6%); Na-diacetate (0.0–0.2%); K-lactate (0.15–5.6%); Na-erythrobate (317 ppm); Na-nitrite (97 ppm); Na-tripolyphosphate (0.276%)

TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

Microorganisms and References	Type of Secondary Model	Response Variables	Independent Variables and Ranges
Gimenez and Dalgaard (in press)	Square-root	μ_{max}	*T* (4–10°C); %WPS (2–6%); smoke components/phenol (3–10 ppm); pH (5.9–6.3); lactic acid (0–20,000 ppm); interaction with lactic acid bacteria
Salmonella			
Gibson et al. (1988)[c]	Polynomial	GT, lag	*T* (10–30°C); NaCl (0.5–4.5%); pH (5.6–6.8); aerobic
Davey and Daughtry (1995)	Arrhenius	Growth rate, lag	Data from Gibson et al. (1988)
Koutsoumanis et al. (1998)	Polynomial	μ_{max}	*T* (22–42°C); pH (5.5–7.0); oleuropein (0–0.8%); aerobic
Oscar (1999)	Polynomial	μ_{max}, lag	*T* (15–40°C); pH (5.2–7.4); previous pH (5.7–8.6); aerobic
Oscar (2002)	Square-root, CPM	μ_{max}, lag	*T* (8–48°C); aerobic
Skandamins et al. (2002)	Vitalistic	Time to decline in cell concentration	*T* (5–20°C); pH (4.3–5.3); oregano essential oil (0.5–2.0%)
Shigella			
Zaika et al. (1994, 1998)[a]	Polynomial	GT, lag	*T* (10–37°C); NaCl (0.5–5.0%); pH (5.0–7.5); Na-nitrite (0–1000 ppm); aerobic
Jeyamkondan et al. (2001)	ANN	GT, lag	Data from Zaika et al. (1994)
Staphylococcus aureus			
Ross and McMeekin (1991)	Square-root	Growth rate	*T* (5–35°C); a_w (0.848–0.997)
Buchanan et al. (1993)[a]	Polynomial	GT, lag	*T* (12–45°C); NaCl (0.5–16.5%); pH (4.5–9.0); Na-nitrite (1–200 ppm); aerobic and anaerobic
Dengremont and Membré (1997)	Square-root	μ_{max}	*T* (10–37°C); NaCl (0–10%); pH (5–8)
Eifert et al. (1997)	Polynomial	Parameters in primary model	*T* (12–28°C); NaCl (0.5–8.5%); pH (5.0–7.0); acidulants HCl, acetic acid, or lactic acid; aerobic
Vibrio spp.			
Miles et al. (1997)	Square-root	GT	*T* (8–45°C); a_w (0.936–0.995); aerobic

TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

Microorganisms and References	Type of Secondary Model	Response Variables	Independent Variables and Ranges
Yersinia spp.			
Bhaduri et al. (1995)[a]	Polynomial	GT, lag	*T* (5–42°C); NaCl (0.5–5.0%); pH (4.5–8.5); Na-nitrite (0–200 ppm); aerobic
Sutherland and Bayliss (1994)[c]	Polynomial	GT, lag	*T* (5–30°C); NaCl (0.5–6.5%); pH (4.0–7.0); aerobic
Pin et al. (2000)	Polynomial	μ_{max}, lag	*T* (1–8°C); CO_2 (0–83%); O_2 (0–60%)
Wei et al. (2001)	Square-root	μ_{max}, lag	*T* (4–34°C); air; vacuum; CO_2 100%

[a] Models included in the Pathogen Modeling Program, which is available free of charge at www.arserrc.gov/mfs/PMP6_download.htm.
[b] Generation time = $\ln(2)/\mu_{max}$.
[c] Model included in Food MicroModel. The values of model parameter are not included in the manuscript.
[d] See Ross et al. (2000) for a list of *Listeria monocytogenes* growth models published prior to 2000.

include germination of spores, population growth, survival, and toxin formation. These types of models became known as "probability" models.

In the latter part of the 1990s it seemed that the only way to manage the risk to consumers from certain pathogens was to ensure that the organism was never present in foods, or to ensure that it was not able to grow in foods that could become contaminated. The latter imperative led to the re-development of "growth/no-growth boundary," or "interface" modeling.

This section is divided into three main parts. In the rst, Section 3.4.2, "traditional" probability modeling is brie y discussed. Section 3.4.3 presents and discusses the newer growth/no-growth (G/NG) modeling approaches, while Section 3.4.4 considers methodological issues relevant to probability and G/NG modeling.

3.4.2 Probability Models

Several reviews of probability modeling were presented in the early 1990s (Baker, 1993; Baker and Genigeorgis, 1993; Dodds, 1993; Lund, 1993; Ross and McMeekin, 1994; Whiting, 1995) but, possibly because of the relative paucity of new publications in this field since then, there has been no more recent dedicated review. Whiting and Oriente (1997) and Zhao et al. (2001), however, provide succinct updates.

TABLE 3.6
Examples of Secondary Models for Growth of Spoilage Microorganisms

Microorganisms and References	Type of Model	Response Variables	Independent Variables and Ranges
Bacillus stearothermophilus			
Ng et al. (2002)	Polynomial	Growth rate, GOL[a]	T (45–60°C); NaCl (0–1.5%); pH (5.5–7.0)
Brochothrix thermosphacta			
McClure et al. (1993)	Polynomial	μ_{max}, lag	T (1–30°C); NaCl (0.5–8.0%); pH (5.6–6.8); aerobic
Abdullah et al. (1994)	Polynomial	GT, lag, MPD	T (−2 to −10°C); CO_2 (2–40%); diameter of meat particles (2–10 mm)
Geeraerd et al. (1998a)	ANN	μ_{max}, lag	Data from McClure et al. (1993)
Pin and Baranyi (1998)	Polynomial	μ_{max}, lag	T (2–11°C); pH (5.2–6.4); aerobic
Koutsoumanis et al. (2000)	Arrhenius	μ_{max}	T (0–20°C); CO_2 (0–100%)
Jeyamkondan et al. (2001)	ANN	GT, lag	Data from McClure et al. (1993)
Chryseomonas spp.			
Membré and Kubaczka (1998)	Square-root	μ_{max}	T (1.3–10°C); aerobic
Enterobacteriaceae			
Pin and Baranyi (1998)	Polynomial	μ_{max}, lag	T (2–11°C); pH (5.2–6.4); aerobic
Lactic acid bacteria			
Passos et al. (1993)	Kinetic	μ_{max}	pH (3.8–6.0); lactic acid (0–30 m*M*); acetic acid (0–40 m*M*); NaCl (0–9%); cucumber juice
Gänzle et al. (1998)	Square-root	μ_{max}	T (3–41°C); aerobic
Gänzle et al. (1998)	CPM	μ_{max}	pH (4.2–6.7); ionic strength (0.0–1.97); acetate (0–0.2 m*M*); aerobic
Devlieghere et al. (2000a,b)	Square-root, polynomial	μ_{max}, lag	T (4–12°C); a_w (0.962–0.9883); CO_2 (0–1986 ppm); Na-lactate (0.0–3.0 5); pH 6.2
Lou and Nakai (2001)	ANN	μ_{max}, lag	T (4–12°C); a_w (0.9 62–0.9883); CO_2 (0–2411 ppm); pH 6.2 Subset of data from Devlieghere et al. (2000a,b)
Wijtzes et al. (2001)	Square-root	μ_{max}	T (3–30°C); a_w (0.932–0.990); pH (5.0–7.5)

TABLE 3.6 (Continued)
Examples of Secondary Models for Growth of Spoilage Microorganisms

Microorganisms and References	Type of Model	Response Variables	Independent Variables and Ranges
Connil et al. (2002)	Polynomial	μ_{max}, lag	T (3–9°C); pH (2.5–6.5); glucose (0.2–0.6%)
Messens et al. (2002)	CPM	Growth, bacteriocin production	T (20–38°C); pH (4.8–7.0)
Leroy and De Vuyst (2003)	CPM	Growth, bacteriocin production	T (20–37°C); pH (4.5–6.5)
Garcia-Gimeno et al. (2002)	ANN	Growth rate, lag	T (20, 28°C); NaCl (0–6%); pH (4–7)
Messens et al. (2002)	CPM	Growth, bacteriocin production	T (20–38°C); pH (4.8–7.0)
Garcia-Gimeno et al. (2002)	ANN	Growth rate, lag	T (20, 28°C); NaCl (0–6%); pH (4–7)
Molds			
Gibson et al. (1994)	Polynomial	Colony growth	T (30°C); a_w (0.810–0.995)
Cuppers et al. (1997)	Square-root, CPM	Colony growth	T (5–37°C); NaCl (0–7%)
Valík et al. (1999)	Polynomial	Colony growth	T (25°C); a_w (0.87–0.995); aerobic
Battey et al. (2001)	Polynomial	Probability of growth	T (25°C); pH (2.8–3.8); titratable acidity (0.2–0.6%); sugar content (8–16°Brix); Na-benzoate (100–350 ppm); K-sorbate (100–350 ppm)
Panagou et al. (2003)	Polynomial, Arrhenius, CPM	Colony growth	T (20–40°C); NaCl (2–10%); pH (3.5–5.0)
Photobacterium phosphoreum			
Dalgaard et al. (1997)[b]	Polynomial, square-root	μ_{max}	T (0–15°C); CO_2 (0–100%)
Pseudomonas			
Membré and Burlot (1994)	Polynomial	μ_{max}, lag	T (4–30°C); pH (6–8); NaCl (0–5%)
Neumeyer et al. (1997)[c]	Square-root	GT	T (0–30°C); a_w (0.947–0.966)
Pin and Baranyi (1998)	Polynomial	μ_{max}, lag	T (2–11°C); pH (5.2–6.4); aerobic

TABLE 3.6 (Continued)
Examples of Secondary Models for Growth of Spoilage Microorganisms

Microorganisms and References	Type of Model	Response Variables	Independent Variables and Ranges
Koutsoumanis et al. (2000)	Arrhenius	μ_{max}	T (0–20°C); CO_2 (0–100%)
Koutsoumanis (2001)	Square-root	μ_{max}, lag	T (0–15°C)
Rasmussen et al. (2002)	Process risk model	GT	Data from Neumeyer et al. (1997)
Shewanella spp.			
Dalgaard (1993)[b]	Square-root	μ_{max}	T (0–35°C); aerobic, anaerobic
Koutsoumanis et al. (2000)	Arrhenius	μ_{max}	T (0–20°C); CO_2 (0–100%)
Yeasts			
Deak and Beuchat (1994)	Polynomial	Changes in conductance	T (10–30°C); a_w (0.93–0.99); pH (3.8–4.6); K-sorbate (0–0.06%)
Passos et al. (1997)	Kinetic with product inhibition	μ_{max}	T (30°C); pH (3.2–5.9); lactic acid (0–55 mM); acetic acid (0–35 mM); NaCl (0–6%); cucumber juice; aerobic and anaerobic
Gänzle et al. (1998)	Square-root	μ_{max}	T (8–36°C); aerobic
Gänzle et al. (1998)	CPM	μ_{max}	Ionic strength (0.0–3.2); acetic acid (0–90 mM); aerobic

[a] GOL = germination, outgrowth, and lag time.

[b] Model included in the Seafood Spoilage Predictor (SSP) software available free of charge at www.dfu.min.dk/micro/ssp/.

[c] Model included in the Food Spoilage Predictor (FSP) software.

3.4.2.1 Logistic Regression

Dodds (1993) explains that in relation to the hazard presented by *Clostridium botulinum* in foods, the detection of the toxin is often more important than growth and that while growth is continuous and fairly easily determined, the presence of detectable toxin was seen as an "all-or-none" response. This led workers to seek methods to predict the probability of production of detectable toxin levels in response to the independent variables.

In probability models in predictive microbiology the data are usually that the response (e.g., growth, detectable toxin production) is observed under the experimental conditions, or that it is not. Responses such as detectable toxin production can be coded as either 0 (response not observed) or 1 (response observed) or, if repeated observations have been made, as probability (between 0 and 1). The probability is related to potential predictor variables by some mathematical function using regression techniques.

Logistic regression is a widely used statistical modeling technique — and is the technique of choice — when the outcome of interest is dichotomous (i.e., has only two possible outcomes). It is widely used in medical research (e.g., Hosmer and Lemeshow, 1989). Because regression techniques do not exist for dichotomous data, the regression equation is usually related to the log odds, or *logit*, of the outcome of interest. This has the effect of transforming the response variable from a binary response to one that extends from $-\infty$ to $+\infty$ re ecting the possible ranges of the predictor variables, and has desirable mathematical features also (Hosmer and Lemeshow, 1989). The logit function is de ned as:

$$\text{logit } P = \log(P/(1 - P)) \tag{3.42}$$

where P is the probability of the outcome of interest.
Logit P is commonly described as some function Y of the explanatory variables, i.e.:

$$\text{logit } P = Y \tag{3.43}$$

Equation 3.43 can be rearranged to:

$$1/(1 + e^{-Y}) = P$$

or

$$e^{Y}/(1 + e^{Y}) = P$$

where Y is the function describing the effects of the independent variables.

The latter parameterizations appear in some of the earlier probability modeling literature.

Zhao et al. (2001) assessed the performance of linear and logistic regression to model percentage data that are "bounded," and may be considered as rescaled probability values. It was con rmed that logistic regression provided a much more accurate description of percentage data than linear regression, which had the insurmountable problem of predicting values outside the range of the data (i.e., less than 0% or greater than 100%).

3.4.2.2 Confounding Factors

Probability modelers used logistic regression to de ne the probability that detectable toxins would be produced within a specified period of time and under specified product composition and storage conditions. Models were based on the idea that a product was safe/acceptable or that it was not. Nonetheless, the responses measured in "probability modeling" were related to a number of factors that were in turn related to the *growth* of the organism under study and, in some cases, also included elements of survival. This approach appears to have arisen from the ideas of Riemann

(1967) that the success of a preservation method with regard to *C. botulinum* is related to the probability that one spore will germinate and give rise to toxin in the nished product. In general, to assess the effect of preservation conditions on probability of toxin production, the probability of growth from a single cell is estimated as the number of spores able to initiate growth under the test conditions (usually determined by MPN [most probable number] methods) divided by the number originally inoculated (Lund, 1993). Often a series of increasingly dilute inocula are subjected to the test conditions to determine the minimum fraction able to initiate detectable growth under the test conditions.

It might be expected that the probability of detection would increase with time. Indeed, Lindroth and Genigeorgis (1986) recognized that the probability of growth detection was also dependent upon the lag time of the inoculum, its initial density, and the duration of the study. They introduced a modi cation to the logit model to specifically model these effects. That model was subsequently used in a number of other studies (Baker et al., 1990; Ikawa and Genigeorgis, 1987). Whiting and Call (1993) criticized earlier models for probability of *C. botulinum* outgrowth and toxin production because they did not specifically monitor the time at which growth/toxin formation was rst detected, and specifically modeled the probability of formation of toxin as a function of time and storage conditions using the logistic function, i.e., the probability of detectable growth, when plotted as a function of time, is a sigmoid curve. That approach was further re ned (Whiting and Oriente, 1997 ; Whiting and Strobaugh, 1998) by inclusion of the inoculum density as an independent variable in the model.

Clearly, the probability of the responses in many of these traditional probability models is strongly related to the growth rate of the organism under the experimental conditions, leading Ross and McMeekin (1994) to conclude that the distinction that had traditionally been made between probability and kinetic models was an arti cial one. Similarly, Baker et al. (1990) noted that "The rate of *P* increase … expresses the growth rate … ."

However, under some experimental conditions *P* does not always reach an asymptote of 1. This is evident in the data of Whiting and colleagues (Whiting and Call, 1993; Whiting and Oriente, 1997; Whiting and Strobaugh, 1998), of Chea et al. (2000), and of Razavilar and Genigeorgis (1998). It had also been described earlier by Lund et al. (1987) who introduced to predictive microbiology a model that recognizes that under some conditions, no matter how long one waits, not all samples will show growth/toxigenesis.

While the above studies considered spores, Razavilar and Genigeorgis (1998) applied a logistic regression approach to the probability of growth initiation within 58 days of *Listeria monocytogenes* and other *Listeria* species in response to combinations of pH, salt, temperature, and methyl paraben, sodium propionate, sodium benzoate, and potassium sorbate (Table 3.5). Their results, also, suggested that under near-growth-limiting environmental conditions the asymptotic probability of growth (i.e., given in nite incubation time) was sometimes less than 1. Stewart et al. (2001) also commented that while kinetic models predict the mean growth rate, these estimates may be meaningless under stressed conditions owing to natural variability in biological responses. Similarly, Lund (1993) employed the

Gompertz model (see Chapter 2) to model the time-dependent probability of growth of *L. monocytogenes* Scott A as a function of environmental factors. Even at near-growth-limiting pH (4.3), however, the asymptote of the log(P_{growth}) was still close to 1.

The above studies suggest that as environmental conditions become more inhibitory to growth, not only does the probability that growth will be observed during the course of the experiment decrease, but the probability that growth *is possible* also decreases. This may be because the generation or lag time of all cells within the inoculum becomes in nitely long. Under these conditions, one begins to identify the absolute limits to microbial growth under combined stresses, i.e., the G/NG interface.

3.4.3 Growth/No Growth Interface Models

Microbial growth is restricted to nite ranges for any environmental factor, with growth rate sometimes declining abruptly within a very small increment of change of environment. Individual factor limits have been determined and collated (e.g., ICMSF, 1996a). That the growth range of microorganisms for one factor is reduced when a second environmental factor is less than optimal is also well recognized, and underlies the Hurdle concept (Leistner et al., 1985) also known as (multiple) barrier technology, or combined processes (Gorris, 2000). While the physiological basis of this synergy remains incompletely understood, the ability to de ne the limits to growth under combined environmental factors has enormous practical application in maintaining the microbial safety and quality of foods. Whether pathogens grow at all and the position of the G/NG boundary are of more interest than their growth rate because any growth implies a potential to cause harm to consumers. Similarly, so-called shelf-stable foods are sold, stored, and consumed over long periods of time. Therefore, the ability of spoilage organisms to grow at all implies that they have the potential to multiply to suf cient numbers to cause spoilage (Jenkins et al., 2000).

In the early to mid-1990s, a vein of experimentation using logistic regression techniques was begun with the aim of developing models that could de ne *absolute* limits to microbial growth in multifactorial space, irrespective of time of incubation or number of cells in the inoculum. One impetus for this research was the problem of listeriosis (Parente et al., 1998; Tienungoon et al., 2000). Strategies proposed to control the threat of listeriosis included "zero tolerance" (i.e., not detectable in a 25-g sample) of the presence of *L. monocytogenes* in foods *that could support its growth*, or to limit levels of contamination *at the point of consumption* to less than 100 cfu/g. Thus, foods that did not support the growth of *L. monocytogenes* were considered to pose signi cantly less risk and to require much less regulatory "attention" and testing. It was, therefore, of great commercial interest to be able to predict, without the need for protracted and expensive challenge testing, the potential for growth of specific bacteria within a particular food or, equivalently, product formulation options that would preclude growth.

Models de ning combinations of environmental conditions that *just* prevent growth have become known as "G/NG interface," "growth boundary," or more simply "growth limits" models. The importance of growth boundary models for the design of safe foods and setting of food safety regulations, for the design of shelf-stable

foods, and as a means of empowering the Hurdle concept by allowing it to be applied quantitatively has been discussed by various authors (Masana and Baranyi, 2000b; McMeekin et al., 2000; Ratkowsky and Ross, 1995; Schaffner and Labuza, 1997). Various approaches have been suggested to define the G/NG boundary. For convenience, these are discussed below under three broad groupings:

1. Empirical, deterministic, approaches
2. Logistic regression techniques
3. Artificial neural networks

Table 3.7 provides an overview of G/NG models published since 1990.

3.4.3.1 Deterministic Approaches

The first explicit definition of a microbial G/NG interface appears to be Pitt (1992), who derived regression equations from published data to describe the temperature/water activity interface for aflatoxin production and *Aspergillus* spp. growth. The equation used to describe the interaction between temperature and water activity limits for growth was:

$$T_g^{(\mathrm{min \cdot max})} = 29.27 \pm \sqrt{(856.71 - 2289 \times (1.172 - a_w))}$$

where $T_g^{(\mathrm{min \cdot max})}$ are the upper and lower temperature limits for growth at the specified water activity.

A similar equation was presented for aflatoxin production. The predicted interfaces from both models are shown in Figure 3.7 .

To describe the pH/$a_{w(\mathrm{NaCl})}$ interface of the food spoilage organism *Brocothrix thermosphacta*, Masana and Baranyi (2000b) derived the midpoints of growth and no-growth observations by interpolation and fitted a polynomial function to those data. They noted that under some conditions, the interface was completely dominated by one factor or the other, so that their final model consisted of a pH vs. a_w parabolic curve and a NaCl-constant line. They also considered the effects of inoculum level on the interface, which was determined at 25°C for up to 24 days of incubation. Examples of the interface are shown in Figure 3.8.

Membré et al. (2001) estimated levels of sorbate that prevented growth of *Penicillium brevicompactum* in bakery products containing various levels of benzoate by extrapolation of kinetic data. Equations were derived to define growth-preventing combinations of sorbate and benzoate and were used to limit the range of predictions from the kinetic model they developed for *P. brevicompactum* growth rate.

Other workers have noted that the form and parameters of CPMs imply absolute limits to microbial growth, and suggested approaches to defining the G/NG interface based on estimates of cardinal parameters. In this vein Ratkowsky and Ross (1995), recognizing the relationship between absolute limits for each environmental factor and their relationship to the parameters of square-root-type models and CPMs, experimented with the use of a kinetic model as the basis of a growth boundary

TABLE 3.7
Summary of Published Growth Boundary Models

			Experimental Design									
					Ranges							
Reference	Organism	Strain	Medium	Environmental Factors	Lower	Upper	Levels	Replicates	Total Data Points	Measured by?	Time Limit	Other
Presser et al. (1998)	*Escherichia coli*	M23 (non-pathogenic)	Nutrient Broth	Temperature	10	37	6	1 to 4	627	OD Increase (confirmed as needed by culture)	50 days	Linear logistic regression, SAS PROCNONLIN
				a_w (NaCl)	0.955	0.995	4					
				pH	2.8	6.9	≥10					
				Lactic acid (mM)	0	500	6					
Parente et al. (1998)	*Listeria monocytogenes*	Scott A, V7, and L11	Tryptone Soy Broth + 0.6% Yeast Extract	Nisin (IU/ml)	1	2100					7 days (@30°C)	Logistic regression with polynomial using LOGIT 1.14 module of Systat
				Leucocidin F10 (AU/ml)	1	2100						
				pH	4.7	6.5						
				NaCl (% w/v)	0.7	4.5						
				EDTA (mmol)	0.1	0.9						
				Inoculum density	1.6×10^3	7.9×10^7						
		Validation Set 1		Nisin (IU/ml)	8	200		5			7 days (@30°C)	
				Leucocidin F10 (AU/ml)	8	200						
				pH	4.7	6.5						
				NaCl (% w/v)	0.7	4.5						
				EDTA (mmol)	0.08	4.72						
				Inoculum density	0.6×10^3	2.5×10^7						
		Validation Set 2		Nisin (IU/ml)	50	250		10			7 days (@30°C)	
				Leucocidin F10 (AU/ml)	1	250						
				pH	5.2	6						
				NaCl (% w/v)	1.8							
				EDTA (mmol)	0.2	0.6						
				Inoculum density	1×10^5							

TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

Reference	Organism	Strain	Medium	Environmental Factors	Ranges Lower	Ranges Upper	Levels	Replicates	Total Data Points	Measured by?	Time Limit	Other
			Experimental Design									
Bolton and Frank (1999)	*Listeria monocytogenes*	Mixture (equal numbers) of Scott A, Brie 1, 71 Switzerland, 2379 LA	Soft fresh cheese (similar to "Mexican style" cheese)	Moisture (% w/w) salt (% w/w) pH	42 2 5	60 8 6.5	4 4 6	3	288	Viable count	21 and 42 days	Binary or "ordinal" logistic regression using SAS PROC LOGISTIC with link functions. For the latter, three responses: P of growth, stasis, or death (according to change in viable count; ± 0.5 log CFU) were modeled
Salter et al. (2000)	*Escherichia coli*	MR21 (STEC)	Nutrient Broth	Temperature a_w (NaCl)	7.7 0.943	37 0.987	60 28	1–8, most 4	604	OD increase	50 days	Nonlinear logistic regression, SAS PROCLOGISTIC and PROCNONLIN
Jenkins et al. (2000)	*Zygosaccharomyces bailii*	4637, history unknown	Acidified yeast nitrogen broth	Salt (NaCl, %w/v) Sugar (fructose, %w/v) Total acetic acid (%v/v) pH	2.6 7 1.8 3.5	4.2 32 2.8 4	3 3 3 3	3	243	OD increase	29 days	SAS LIFEREG

Tienungoon et al. (2000)	*Listeria monocytogenes*	(Scott A, L5 separate models)	TSB-YE	Temperature	3.1	36.2	30	1 to 4	2839	OD increase	90 days	Nonlinear logistic regression, SAS PROCLOGISTIC and PROCNONLIN
				a_w (NaCl)	0.928	0.995	60					
				pH	3.7	7.8	10					
				Lactic acid (mM)	0	500	14					
López-Malo et al. (2000)	*Saccharomyces cerevisiae*	Not stated Model based on data of Cerruti et al. (1990)		a_w	0.93	0.97	3		72 (× 2 observation times)	Viable count, including decrease in viable count	50 h or 350 h	Logistic regression with rst-order polynomial using SPSS
				pH	3	6	4					
				K-sorbate (ppm)	0	1000	6					
Masana and Baranyi (2000b)	*Brocothrix thermosphacta*	MR 165	Tryptone Soya Broth	NaCl	0.5	10	11			Viable count		Polynomial fitted to midpoints of data-pairs of adjacent growth and no growth observed combinations
				pH	4.4	5.7	7					
				Inoculum (cfu/ 350 µl)	10	1 million	3					
		Fine grid experiments		NaCl pH	45 combinations at 5 levels of NaCl and 5 levels of pH close to the G/NG boundary			10	450	Viable count		
				Inoculum (cfu/ 350 µl)	10	1000	2					
Lanciotti et al. (2001)	*Bacillus cereus*	FG1	BHI Broth	Temperature	10	45	5	30 variables combinations over two independent trials for each organism	2 × 30 for each strain	OD increase (600nm)	2–7 days	Generalized linear logistic regression, Statistica (Statsoft) software
	Staphylococcus aureus	S33	BHI Broth	a_w (glycerol)	0.89	0.99	8					
	Salmonella enteritidis	B5	BHI Broth	pH	4	8	5					
				Ethanol (% v/v)	0	3	5					

TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

Reference	Organism	Strain	Medium	Environmental Factors	Ranges: Lower	Ranges: Upper	Levels	Replicates	Total Data Points	Measured by?	Time Limit	Other
			Experimental Design									
Stewart et al. (2001)	*Staphylococcus aureus*	5 strain cocktail	BHI Broth	a_w (glycerol)	0.95	0.84	4	8	640	OD increase	168 days	Toxin assayed Modeled "time to growth" using LIFEREG
				Initial pH	4.5	7	4					
				K-sorbate (ppm) or								
				Ca-propionate (ppm)	0	1000	3					
				Temperature (°C)	37							
McKellar and Lu (2001)	*Escherichia coli* O15:H7	5 strain cocktail	TS Broth	Temperature (°C)	10	30	5	5	1820	Visible increase in turbidity	3 days	Linear logistic regression used (polynomial form)
				Acetic acid (modeled as undissociated form)	0	4%	8					
				NaCl	0.50%	16.50%	8					
				Sucrose	0	8%	3					
				pH	3.5	6.0	6					

Membré et al. (2001)	*Penicillium compactum*	Wild type from bakery products	MY50 agar	pH	2.5	7.5	± 6	1	76	Mycelial growth	75 days	Not directly modeled, growth limits due to sodium benzoate and sorbic acid at pH 5 were derived by extrapolation of growth rate data
				Sorbic acid			1					
				Propionic acid			1					
				Sodium benzoate			1					
				pH			1	5	122			
				Sorbic acid (mg l^{-1})	0.0	1000.0	6					
				Na-benzoate (mg l^{-1})	0.0	300.0	4					
			Commercial cakes					6	4			
Uljas et al. (2001)	*Escherichia coli* O15:H7	3 strain cocktail	Apple cider (juice)	pH	3.1	4.3	7		1600 × 3	Turbidity (growth within 48 h at 35°C) after dilution of treated sample	12 hours	SAS PROCLOGISTIC (dependency modeled as simple first order equations of predictor variables, no cross products)
				Temperature	5	35	4					
In this case the response modeled was P > 5 log inactivation after various treatment times				Sodium benzoate	0	0.1%	3					
				Potassium sorbate	0	0.1%	3					
				Freeze–thaw	Not applied	Applied	3	756				
				Ciders type			3	2 or 3				
	Staphylococcus aureus	5 strain cocktail	BHI Broth	a_w (NaCl, or sucrose-fructose)	0.84	0.95	4 2	8		OD increase	168 days	Toxin assayed Modeled "time to growth" using LIFEREG
				pH	4.5	7	4					
				K-sorbate (ppm)	0	1000	2					
		[Combined with data set of Stewart et al. (2001), 768 data]							1792			
	Listeria innocua	ATCC 33090	BHI Broth (+0.2% w/w glucose, +0.3% w/w yeast extract	Temperature	0	43	16	(pH constant)		Turbidimetry	14 days	Novel term based on relative inhibition of growth rate-affecting factors data generated for combined kinetic model that predicts no growth (NLINFIT in MATLAB 5.2)
				pH	4.5	9.4	15	(temperature constant)		Viable count by culture	1 month	
				Propionic acid (mM)	16	64	24	(pH varied from 5 to 7.5)				
				Lactic acid (mM)	20	138	27	(pH varied from 4.8 to 7.1)				

TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

Reference	Organism	Strain	Medium	Experimental Design: Environmental Factors	Ranges: Lower	Ranges: Upper	Levels	Replicates	Total Data Points	Measured by?	Time Limit	Other
Battey and Schaffner (2001)	Spoilage bacteria: *Acinetobacter calcoaceticus* and *Gluconobacter oxydans*	2 strain cocktail	Cold lled, ready to drink, beverages	pH	3	2.8	3.8	84		Viable plate count	8 weeks at 25°C	Model is based on growth and inactivation rates. Included 14 duplicated validation trials (8 correctly predicted from model)
				Titratable acidity (%)	3	0.2	0.6					
				Sodium benzoate (ppm)	3	100	350					
				Sugar content (°C Brix)	3	8	16					
				Potassium sorbate (pp)	3	100	350					
Battey et al. (2002)	Spoilage yeasts: *Saccharomyces cerevisiae, Zygosaccharomyces bailii, Candida lipolytica*	3 strain cocktail	Cold lled, ready to drink beverages	pH	3	2.8	3.8	84		Viable plate count	8 weeks at 25°C	Included 14 duplicated validation trials (all correctly predicted from model)
				Titratable acidity (%)	3	0.2	0.6					
				Sodium benzoate (ppm)	3	100	350					
				Sugar content (°C Brix)	3	8	16					
				Potassium sorbate (pp)	3	100	350					
Hajmeer and Basheer (2002, 2003a, 2003b)	Data of Salter et al. (2000), see above											Probabilistic Neural Network

model using linear logistic regression. This approach is discussed further below in Section 3.4.3.2.

The approaches of Augustin and Carlier (2000b) and Le Marc et al. (2002) were presented in Section 3.2.3. Essentially, these approaches are empirical. They are based on assumed interactions between factors and are not *fitted* to G/NG data. An example of the response predicted by these approaches is shown in Figure 3.11.

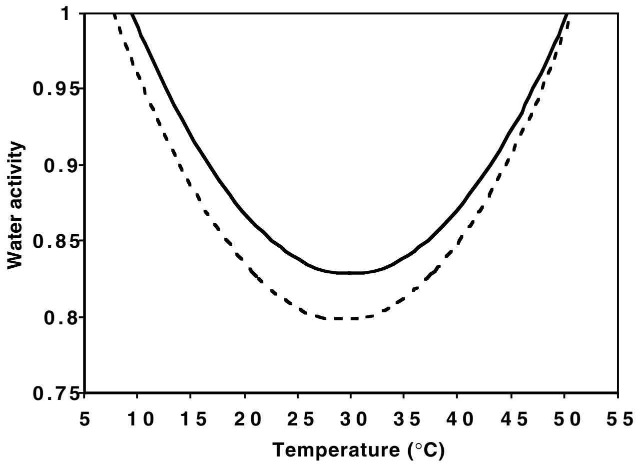


FIGURE 3.7 Predicted temperature–water activity interface for mold (*Aspergillus* spp.) growth (dashed line) and a atoxin production (solid line) from the model of Pitt (1992).

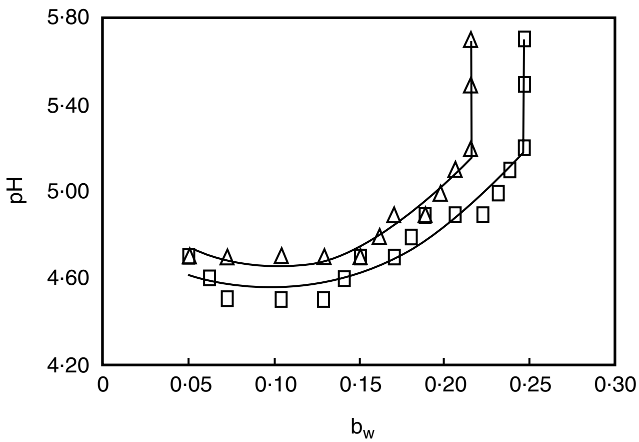


FIGURE 3.8 Data and modeled growth/no-growth boundary for *Brochothrix thermosphacta* in response to pH and water activity at 25°C. Water activity data were rescaled to $b_w = \sqrt{1 - a_w}$. The data are for an inoculum of 1.5×10^6 cells/well (□), or for an inoculum of 1.5×10^1 and 1.5×10^3 cells/well (△). (Reproduced from Masana, M.O. and Baranyi, J. *Food Microbiol.*, 17, 485–493, 2000b. With permission.)

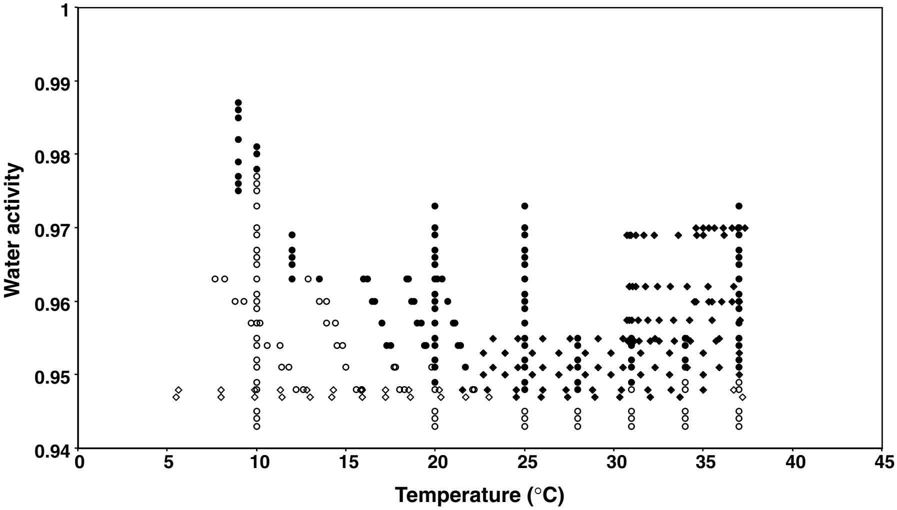


FIGURE 3.9 Data obtained from separate experiments for the growth/no-growth (G/NG) boundary of *Escherichia coli.* Data are from Salter et al. (2000) (circles) and from unpublished results of the authors (diamonds). Near the G/NG boundary, the data obtained from discrete experiments do not form a smooth (monotonic) boundary, suggesting that small differences in experimental procedures can signi cantly affect the position of the boundary. Open symbols denote no-growth conditions, and solid symbols indicate that growth was observed.

The above approaches can be considered to be deterministic; i.e., they predict only one position ($P_{growth} = 0.5$) for the boundary, although the position of boundaries can be adjusted by "weighting" data in the case of Masana and Baranyi (2000b) or by selecting an appropriate value for θ in the case of the Le Marc et al. (2002) approach (see Section 3.2.3). While the data of Masana and Baranyi (2000b) included tenfold replication, the midpoints of the *most* "extreme" conditions that *did* allow growth, and the *least* "extreme" conditions that *did not* allow growth were estimated by interpolation and considered to represent 50% probability of growth. Other workers have suggested that some problems require higher levels of con dence that growth will not occur, so that methods that enable de nition of the interface at selected levels of statistical con dence may have greater utility.

Another approach that implicitly characterizes the G/NG interface is that of combined growth and death models in which the rate of growth and rate of death under specified conditions are estimated simultaneously. The G/NG interface can be inferred from those combinations of conditions where growth rate and death rate are equal (see, e.g., Jones and Walker, 1993; Jones et al., 1994). A similar approach is evident in Battey et al. (2001) who modeled both the rates of growth and rates of death of spoilage molds in ready to drink beverages. The G/NG interface was given, implicitly, as that set of conditions where the rate of growth was equal to the rate of death.

Ratkowsky et al. (1991) noted that as environmental conditions become more inhibitory to microbial growth the variability in growth rates increases widely, which implies that the probability that growth occurs at all becomes uncertain, because the left-hand tail of the growth rate distribution falls below zero. This is supported in the results of Whiting and colleagues (Whiting and Call, 1993; Whiting and Oriente, 1997; Whiting and Strobaugh, 1998), where P_{max} (the proportion of spores that successfully germinated and initiated growth) was shown to decline at increasingly stringent conditions. Conversely, Masana and Baranyi (2000b) observed, as have other workers (McKellar and Lu, 2001; Presser et al., 1998; Salter et al., 2000; Tienungoon et al., 2000), that the difference in conditions that allow growth and those that do not is usually abrupt, and often at or beyond the limits of resolution of instruments commonly used to measure such differences. Thus, Masana and Baranyi (2000b) questioned the need for approaches that model the transition between conditions leading to high probability of growth and those leading to low probabilities of growth. While this abrupt transition appears consistent *within* replicated experiments it is less certain, however, that the same consistency is true *between* experiments. Figure 3.9, showing experimental data, suggests that responses near the boundary may be inconsistent when data from several discrete experiments are combined. This may suggest subtle, but important, differences in response related to the physiology of the inoculum, or its concentration. Furthermore, it suggests that the ability to characterize probabilities of growth under specified sets of conditions may be an important element of growth boundary models and that the boundary may not be "absolute" but depend on the physiological state of the cell and, by inference, on the size of the inoculum. This will be discussed further in Section 3.4.4.

3.4.3.2 Logistic Regression

Ratkowsky and Ross (1995) and others (Bolton and Frank, 1999; Jenkins et al., 2000; Lanciotti et al., 2001; López-Malo et al., 2000; McKellar and Lu, 2001; Parente et al., 1998; Stewart et al., 2001, 2002; Uljas et al., 2001) reintroduced the use of logistic regression to model categorical data (i.e., growth or no growth) in predictive microbiology, enabling probabilistic determination of the G/NG boundary. Use of the logit function enabled the probability of growth under specific sets of conditions to be estimated, so that the G/NG boundary could be specified at selected levels of con dence.

Ratkowsky and Ross (1995) aimed to model absolute limits to growth in multifactorial space, but only had available data based on a 72-h observation period. While most other workers have preferred polynomial functions to describe the effect of independent variables on the logit function, in the former approach a square-root-type kinetic model was ln-transformed and used as the basis of the function relating the logit of probability of growth to independent variables, e.g., temperature, water activity, pH. This approach was adopted in an attempt to retain some level of biological interpretability of the models, a desire echoed by others (Augustin and Carlier, 2000a,b; Le Marc et al., 2002).

The form of the G/NG interface model of Presser et al. (1998) was derived from the kinetic model of Presser et al. (1997) for the growth rate of *E. coli* (see Equation

3.10). Novel data were generated specifically to assess the limits of *E. coli* growth under combinations of temperature, pH, a_w, and lactic acid. The corresponding G/NG model had the form:

$$\begin{aligned}\text{LogitP} = {} & 28.0 + 8.90 \ln(a_w - 0.943) \\ & + 2.0\ln(T\text{-}4.00) + 4.59 \ln(1 - 10^{3.9\text{-pH}}) \\ & + 6.96\ln[1 - LAC/(10.7 \times (1 + 10^{\text{pH-}3.86}))] \\ & + 3.06\ln[1 - LAC/(823 \times (1 + 10^{3.86\text{-pH}}))]\end{aligned} \tag{3.44}$$

where all terms are as de ned in Section 3.2.1.

Some parameters in that model had to be determined independently, i.e., were not determined in the regression, and were derived from the fitted values of square-root-type kinetic models. Essentially the same approach was adopted by Lanciotti et al. (2001) to develop G/NG models for *B. cereus*, *S. aureus,* and *Salmonella enteritidis*. Ratkowsky (2002) commented on the increased exibility in being able to determine all of the parameters in the model during the regression, and subsequent studies developed the approach, eventually leading to a novel nonlinear logistic regression technique (Salter et al., 2000; Tienungoon et al., 2000). Ratkowsky (2002) pointed out that nonlinear logistic regression was a new statistical technique and discussed bene ts and problems with that approach specifically in relation to growth limits modeling. A problem with models of the form of Equation 3.44 is that for conditions more extreme than the parameters corresponding to T_{min}, pH_{min}, $a_{w\,min}$, etc., and which are tested experimentally though not expected to permit growth, the terms containing those parameters would become negative. As all of those terms are associated with a logarithmic transformation, the expression cannot be calculated during the regression and such values are ignored in the model tting process, or have to be eliminated from the data set before the tting process begins. This, in turn, affects the values of the parameters of the fitted model. Ratkowsky (2002) comments that an objective method for selection and deletion of such data is necessary, but does not yet exist.

Bolton and Frank (1999) extended the binary logistic regression approach by recoding growth and no growth data to allow a third category: survival, or stasis. They termed this approach ordinal logistic regression. Parente et al. (1998) "reversed" the response variable, and applied logistic regression techniques to the probability of survival/no survival of *L. monocytogenes* in response to bacteriocins, pH, EDTA, and NaCl. Stewart et al. (2001) modeled the probability of growth of *S. aureus* within 6 months of incubation at 37°C, and at reduced water activity achieved by various humectants. They also compared the growth boundary with the boundary for enterotoxin production, and observed a close correlation between the two criteria.

Growth limits models have also been developed for spoilage organisms including *Saccharomyces cerevisiae* (López-Malo et al., 2000) and *Zygosaccharomyces bailii* (Jenkins et al., 2000) and cocktails of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Candida lipolytica* (Battey et al., 2002). Interestingly, the study of Jenkins et al. (2000), while encompassing broader ranges of factor combinations, con rmed

the simpler and earlier model of Tuynenburg Muys (1971). That model, which specifies combinations of molar salt plus sugar and percent undissociated acetic acid for stability of acidic sauces, still forms the basis of the industry standard for those products. This observation suggests that limits to growth under combined conditions can be highly reproducible.

3.4.3.3 Relationship to the Minimum Convex Polyhedron Approach

The concept of the MCP was introduced by Baranyi et al. (1996) (see Figure 3.10) to describe the multifactor "space" that just encloses the interpolation region of a predictive kinetic model. If the interpolation region exactly matched the growth region of the organism then the MCP would also describe the growth limits of the organism. In practice, however, it would be impossible to undertake suf cient measurements to completely de ne the MCP; i.e., the MCP has "sharp" edges because of the method of its calculation, whereas from available studies (see Figures 3.8 and 3.9) the G/NG interface forms a continuously curved surface. However, it might also be possible to use no-growth data to create a no-growth MCP and to combine the growth MCP and no-growth MCP to de ne a region within which the G/NG boundary must lie. This approach has been assessed and compared to a model of the form of Equation 3.43 by Le Marc and colleagues (Le Marc et al., 2003). These workers concluded that the logistic regression modeling approach produced a smoother response surface, more consistent with observations, but that the MCP approach had the advantage of being directly linked to observations and therefore was not a prediction from a model.

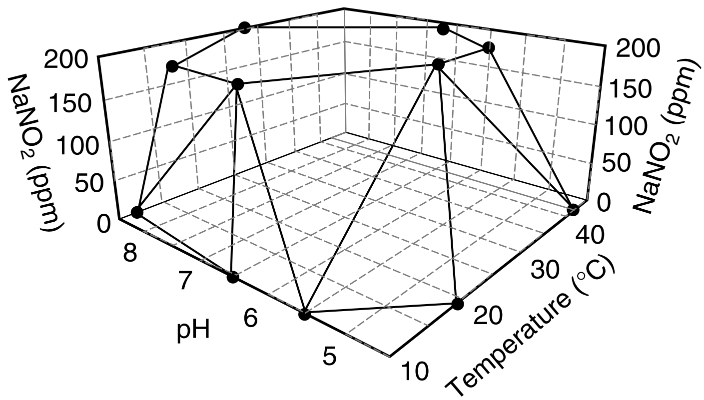


FIGURE 3.10 Interpolation region (MCP) for a model that includes four-factor combinations (T, pH, NaCl, $NaNO_2$). The interpolation region shown is that for NaCl = 0.5%, but is based on the complete data set. Solid circles indicate conditions under which observations have been made, while the lines represent the edges of the MCP. (From Masana, M.O. and Baranyi, J. *Food Microbiol.*, 17, 367–374, 2000a. With permission.)

3.4.3.4 Artificial Neural Networks

Recently, Hajmeer and Basheer (2002, 2003a,b) demonstrated the use of a Probabilistic Neural Network (PNN) approach to definition of the G/NG interface. PNNs are a form of ANN (see Section 3.2.6). In a series of papers, based on modeling the data of Salter et al. (2000) for the effects of temperature and water activity (due to NaCl) on the growth limits of *E. coli,* Hajmeer and Basheer concluded that their PNN models provided a better description of the data of Salter et al. (2000) than did the nonlinear logistic regression method referred to above. Their conclusion is considered in more detail in Section 3.4.3.5 below.

It should be noted that neither the logistic regression models described above, nor the PNN, produce an equation that describes the interface. Rather, the output of those models is the probability that a given set of conditions will allow growth. To define the interface, it is necessary to rearrange the model for some selected value of *P* to generate an equation that describes the G/NG boundary.

3.4.3.5 Evaluation of Goodness of Fit and Comparison of Models

Methods for evaluation of performance of logistic regression models include the receiver operating curve (ROC; also referred to as the concordance rate), the Hosmer–Lemeshow goodness-of-fit statistic, and the maximum rescaled R^2 statistic. These are considered in greater detail in Tienungoon et al. (2000).

Briefly, the ROC is obtained from the proportion of events that were correctly predicted compared to the proportion of nonevents that were correctly predicted. The closer the value is to 1, the better the level of discrimination. In epidemiological studies, ROC values > 0.8 are considered excellent. ROC values for G/NG models are typically much higher.

The Hosmer–Lemeshow index involves grouping objects into a contingency table and calculating a Pearson chi-square statistic. Small values of the index indicate a good fit of the model.

The maximum rescaled R^2 value is proposed for use with binomial error as an analogy to the R^2 value used with normally distributed error. The closer the value is to 1, the greater is the success of the model in predicting the observed outcome from the independent variables. Zhao et al. (2001) cite the deviance test and graphical tools such as the index plot and half normal plot as methods for determining goodness of fit of linear logistic regression models.

Other methods based on calculation of indices from the "confusion matrix" (Hajmeer and Basheer, 2002, 2003b) or the equivalent "contingency matrix" (Hajmeer and Basheer, 2003a) were used to compare performance between models derived from different approaches and applied to the same data.

Another method of evaluation is to compare the fitted model to independent data sets (Bolton and Frank, 1999; Masana and Baranyi, 2000b; Tienungoon et al., 2000) although, generally, such data are not readily available (see, e.g., McKellar and Lu, 2001). The model of Tienungoon et al. (2000) for *L. monocytogenes* growth boundaries showed very good agreement with the data of McClure et al. (1989) and George

et al. (1988) despite that different strains were involved. There is also a remarkable level of similarity between the observations of Tienungoon et al. (2000) and the observations of Le Marc et al. (2002) on growth limits of *L. innocua*. Several publications, however, report growth of *L. monocytogenes* at temperatures lower than the minimum growth limit predicted by the Tienungoon et al. (2000) model, possibly indicating strain variation or that the experimental design failed to recognize important elements that facilitate *L. monocytogenes* growth at temperatures < 3°C, i.e., that an inappropriate growth substrate was used. Similarly, McKellar and Lu (2001) reported that their model failed to predict growth of *E. coli* O157:H7 under conditions where it had been previously reported, although it should be noted that their model was limited to observation of growth within 72 h. Bolton and Frank (1999) compared the predictions of their growth limits models for *L. monocytogenes* in cheese to the data of Genigeorgis et al. (1991) for *L. monocytogenes* growth in market cheese. The models predicted correctly in 65% of trials (42-day model) and 81% of trials (21-day model).

Given the diversity of approaches, it is pertinent to ask: does one method for de ning the G/NG interface perform better than another? As with kinetic models, the ability to describe a specific experimental data set does not necessarily reflect the ability to predict accurately the probability of growth under novel sets of conditions. While measures of performance of logistic regression models are available, they can be readily affected by the data set used. Perfect agreement between observed and modeled data responses may not be possible if there are anomalies in the data. Figure 3.11 provides a clear example. Nonetheless, for many growth limits models high rates of concordance (typically >90%) have been reported. As noted earlier, in epidemiological logistic regression modeling, rates higher than 70% are considered to represent good ts to the data, implying that the limits to microbial growth are highly reproducible when well-controlled experiments are conducted.

To date, only one direct comparison of G/NG modeling approaches has been presented (Hajmeer and Basheer, 2002, 2003a,b) but this was based on one data set only, i.e., that of Salter et al. (2000) for the growth limits of *E. coli* in temperature/water activity space. Only by comparing the performance of different modeling approaches applied to multiple data sets does an appreciation of overall model performance emerge. Nonetheless, to illustrate differences between models and give some appreciation of their overall performance we compare several models using the data of Salter et al. (2000) for the growth limits of *E. coli* R31 in response to temperature and water activity. The model types compared are:

1. The PNN of Hajmeer and Basheer (2003a), which those authors were able to summarize as a relatively simple equation
2. A model of the type of Equation 3.44 fitted to a subset of the Salter et al. (2000) data set by Hajmeer and Basheer (2003a) (It should be noted that, contrary to what is stated in that publication, the model presented by Hajmeer and Basheer was not generated by nonlinear logistic regression but by a two-step linear logistic regression as described in Presser et al., 1998)

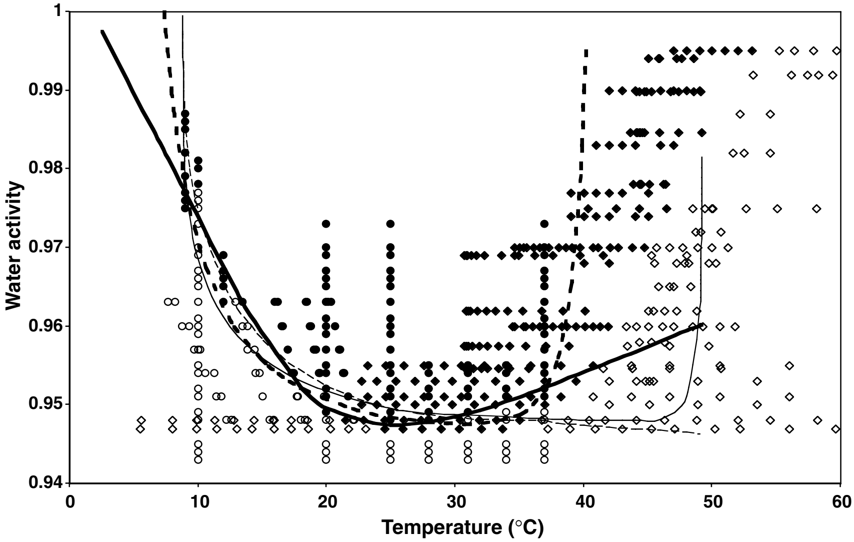


FIGURE 3.11 Comparison of predicted no growth boundaries for four modeling approaches applied to the data of Salter et al. (2000) (circles) for the growth limits of *Escherichia coli* R31 in response to temperature and water activity (NaCl) combinations. Approach of Hajmeer and Basheer PNN (heavy solid line); Linear Logistic /Equation 3.44 (heavy dashed line); Le Marc et al. (2002) (light solid line); Augustin and Carlier (2000a) (light dashed line). The data set was subsequently augmented with new data (diamonds), which reveals that none of the models extrapolate reliably. (Solid symbols: growth; open symbols: no growth.)

3. A model of the type of Le Marc et al. (2002; Equation 3.25 to Equation 3.27), where T_{max} = 49.23°C (to be consistent with the logistic regression model parameter), $a_{w\,min}$ = 0.948, and T_{min} = 8.8°C, based on the minimum water activity and temperature, respectively, at which growth were observed
4. A model of the type of Augustin and Carlier (2000b; Equation 3.24) assuming that T_{min} = 8.8°C and $a_{w\,min}$ = 0.948, consistent with the parameter values used for the Le Marc et al. (2002) model

The predicted interfaces are shown in Figure 3.11, together with the data used to generate the models. (Note that the subsets of 143 of the 179 data of Salter et al. (2000) used by Hajmeer and Basheer (2003a) to t the PNN and the Equation 3.44 type model were not identi ed.)

When compared to the full data set, the level of misprediction ranged from ~15 to 20 of the 179 data points for each of the models, suggesting that the level of performance was not greatly different despite very different modeling approaches.* A complication in the comparison of G/NG model performance is that most of the

* It should be noted that this analysis disagrees with the results of Hajmeer and Basheer (2002) who reported only two to four mispredictions for the total (i.e., training and validation) data set.

data are readily predicted, e.g., those that fall outside the known limits for growth for individual environmental factors when all other factors are optimal. Such data can "overwhelm" the data in which one is most interested, i.e., in the relatively narrow region where factors interact to reduce the biokinetic ranges and, yet more specifically, where the probability of growth rapidly changes from "growth is very likely" to "growth is very unlikely." These data define the interface and, consequently, data closest to the interface are more important when comparing model performance. This has implications for experimental design, as discussed in Section 3.4.4 below.

To assess whether one model might be preferred on theoretical grounds, as adjudged by its ability to extrapolate reliably, the predictions of all models in the temperature range above 35°C can be compared to data subsequently generated, shown in Figure 3.11, and not used to generate the models. Clearly, none of the models extrapolate well.

From the above comparison, it appears that despite very different modeling approaches and degrees of complexity of modeling, there is currently little to differentiate those approaches on the basis of their ability to describe the G/NG interface or on their ability to predict outside the interpolation region.

3.4.4 Experimental Methods and Design Considerations

As suggested above, currently there is little mechanistic understanding of how environmental factors interact to prevent bacterial growth and it must be recognized as a possibility that there is no single, common mechanism underlying the observed boundaries for different factor combinations. Consequently, it is not possible from first principles to design the optimal experiment that captures the essential information that will characterize the response and lead to reliable models. Instead, at this time, experimental methods must be focused toward gaining enough data in the interface region to be able to describe empirically the limits to growth.

First of all, two approaches may be distinguished that could affect the experimental methods chosen. In one, the interest is in whether growth/toxin production, etc. is possible within some specified time limit, which may be related to the shelf life of the product. In other approaches, the objective is to define absolute limits to growth, i.e., the most extreme combinations of factors that just allow growth. McKellar and Lu (2001) argue that there is always a time limit imposed on G/NG modeling studies. Strategies exist, however, that provide greater confidence that the "absolute" limits to growth are being measured. Some of these are discussed below.

3.4.4.1 Measuring Both Growth and Inactivation

Several groups have assessed both growth and inactivation in their experimental treatments (McKellar and Lu, 2001; Parente et al., 1998; Presser et al., 1999; Razavilar and Genigeorgis, 1998). In this way the position of the boundary is inferred from two "directions." If growth is not observed, an observer cannot be sure whether growth is not possible or has not occurred *yet*. If it is known that at some more extreme condition inactivation occurs, it can be inferred that the G/NG boundary lies between those two sets of conditions.

A potential problem with this strategy is that cultures can initially display some loss of viability, but with survivors eventually initiating growth; i.e., population decline cannot unambiguously be interpreted as "growth is not possible." Numerous studies (e.g., Mellefont et al., 2003) have demonstrated that rapid transfer of a culture from one set of conditions to another that is more stressful can induce injury and death, but that survivors will eventually adjust and be able to grow. This has been termed the Phoenix phenomenon (Shoemaker and Pierson, 1976). Such regrowth has been reported in the context of G/NG modeling (Bolton and Frank, 1999; Masana and Baranyi, 2000b; Parente et al., 1998; Tienungoon et al., 2000).

Clearly, an experimenter interested in determining the "absolute" G/NG boundary will need to maximize the resistance of the inoculum to stress on exposure to the new, more stressful, environment. The use of stationary phase cultures as inocula would seem to be a minimum requirement. It may be necessary to habituate cultures to the test conditions (e.g., growth at conditions just less harsh) prior to inoculation into the test conditions to maximize the chance that growth, if possible, will be observed. One way to maximize the likelihood of observing the most extreme growth limits would be to use cultures growing at the apparent limits as inocula into slightly more stringent conditions. This also has the advantage of minimizing growth lags on inoculation into a harsher environment.

3.4.4.2 Inoculum Size

Masana and Baranyi (2000b) indicated that inoculum size affected the position of the boundary. Robinson et al. (1998) reported similar effects of inoculum density on bacterial lag times. While it is clear that time to detection would depend on inoculum density, growth detection methods were not cell-density-dependent in those studies. Parente et al. (1998) also reported that a decrease in inoculum size decreased the probability of survival. If the shock of transfer is known to inactivate a fixed proportion of the cells in the inoculum, to develop a robust model it will be necessary to use an inoculum that ensures that even after inactivation there is a high probability that at least one cell will survive.

The above observations lend support to the hypothesis that it is the distribution of physiological states of readiness to survive and multiply in a new environment that determines the position of the G/NG boundary, i.e., all other things being equal, the more cells in the inoculum the more likely it is that there is one cell that has the capacity to survive and grow. This also reinforces the equivalence between G/NG boundary modeling and the modeling of conditions that lead to infinite lag times. The importance of the distribution of lag times on the observed lag times of whole populations has been discussed by Baranyi (1998).

There may be more involved reasons for inoculum density-dependent responses also, such as chemical messaging between cells (see, e.g., Miller and Bassler, 2001; Winans and Bassler, 2000).

In conclusion, if the aim is to determine absolute limits to growth, a higher number of cells is preferable. Masana and Baranyi (2000b) stated that growth boundaries "represented the chance of growth for each sample; therefore, to assure a low probability of growth in many samples, it will be more relevant to consider

boundaries for high inoculum levels." Equally, as noted above, steps to maximize the cell's chances of survival and growth in the environment are also recommended.

There is potentially a caveat, however, that needs to be applied. Maximum population densities of batch cultures are reported to decline under increasingly harsh growth conditions. Thus, the use of high inocula may mask the true position of the G/NG boundary if the inoculum used is already denser than the MPD of the organism in a very stressful test environment.

3.4.4.3 Are There Absolute Limits to Microbial Growth?

In the above discussion it has been implicitly assumed that there are absolute limits to microbial growth under combined environmental stresses. It is pertinent to examine this assumption.

Numerous authors have noted that, within an experiment, the transition between conditions that allow growth, and those that do not, is abrupt and that usually all replicates at the last growth condition grow, while all the replicates at the rst-growth-preventing condition do not (Masana and Baranyi, 2000; Presser et al., 1998; Tienungoon et al., 2000). McKellar and Xu (2001), for example, reported that of 1820 conditions tested, all ve replicates of each condition either grew or did not grow. This abruptness, however, is not always evident in the modeled results (Tienungoon et al., 2000).

Conversely, between experiments by the same researcher, using the same methods and the same strain, results are not always reproducible. Figure 3.9 provides an example and Masana and Baranyi (2000b) make the same observation of their data for *Brochothrix thermosphacta*. At the same time, however, there is evidence of excellent reproducibility of boundaries between independent workers, using different strains, and different methods in different locations. The results of Tienungoon et al. (2000) were highly consistent between two strains tested, and more notably, with those of George et al. (1988) and Cole et al. (1990) presented a decade earlier, including different strains in one case. There is also a remarkable similarity between the pH/temperature G/NG interface of *Listeria innocua* reported by Le Marc et al. (2002) and the same interface for two species of *L. monocytogenes* presented in Tienungoon et al. (2000).

Jenkins et al. (2000) noted that the boundary they derived for the growth limits of *Zygosaccharomyces baillii* in beverages was very consistent with a model developed 30 years earlier for the stability of acidic sauces.

Stewart et al. (2002) noted that with *S. aureus*, as conditions became increasingly unfavorable for growth, the contour lines (P_{growth}) they generated drew closer and closer together, suggesting that conditions were approaching absolute limits that do not allow growth. Conversely, there are examples where one group's observations do not agree well with another's for an analogous organisms/environmental pair (e.g., Bolton and Frank, 1999; McKellar and Xu, 2001). Delignette-Muller and Rosso (2000) reported strain variability in the minimum temperature for growth.

While the above discussion points to heterogeneity in the physiological readiness of bacteria to grow in a new environment, Masana and Baranyi (2000b) also infer that differences in microenvironments, particularly within foods, could also be a source of heterogeneity in observed growth limits.

In conclusion, there is a body of experimental evidence that suggests that growth boundaries, if carefully determined, might be highly reproducible. Conversely, counterexamples exist. It remains to be determined whether the incongruous results arise from signi cant and measurable differences in methodology, e.g., the detection time used in the respective studies, or are due to uncontrollable sources (Table 3.7).

3.4.4.4 Experimental Design

As noted above, it is not possible from rst principles to design the optimal experiment that captures the information to characterize the G/NG boundary. Various authors have suggested physiological interpretations (Battey et al, 2001; Battey and Schaffner, 2001; Jenkins et al., 2000; López-Malo et al., 2000; McMeekin et al., 2000) but none have yet been experimentally tested.

Thus, an empirical approach that aims to collect as much information in the region of most interest, i.e., the G/NG interface, is recommended by most workers. Several groups of researchers have indicated that they use a two-stage modeling process. The rst uses a coarse grid of conditions of variables to roughly establish the position of the boundary. The second phase monitors responses at conditions near the boundary and at close intervals of the environmental parameters. Variable combinations far from the interface, at which growth is either highly likely or highly unlikely, do not provide much information to the modeling process, which seeks to de ne the interface with a high level of precision. Equally, it is ideal to use a design that gives roughly equal numbers of conditions where growth is, and growth is not, observed (Jenkins et al., 2000; Legan et al., 2002; Masana and Baranyi, 2000b, Uljas et al., 2001). Pragmatically, Legan et al. (2002) recommend setting up "marginal" and "no-growth" treatments rst because these treatments will run for the longest time (possibly several months). Those conditions in which growth is expected to be relatively quick can be set up last because they only need monitoring until growth is detected.

The nature of these studies necessarily involves long incubation times. Legan et al. (2002) noted that particular care must be taken to ensure that the initial conditions do not change over time solely as a result of an uncontrolled interaction with the laboratory environment. Prevention of dessication or uptake of water vapor requires particular attention. Changes resulting from microbial activity may, however, be an important part of the mechanism leading to growth initiation and should not be stabilized at the expense of growth that would naturally occur in a food. Legan et al. (2002) comment that, for example, maintaining the initial pH over time is typically neither possible nor practical, even in buffered media, and that allowing a change in pH due to growth of the organism more closely mimics what would happen in a food product than maintaining the initial pH over time.

3.4.4.5 Conclusion

From the above discussion, unambiguous de nition of the G/NG boundary of an organism in multidimensional space presents several paradoxical challenges. While an experimenter will do well to remember these considerations in the interpretation

of his/her results, it seems probable that methods that have been used to date will have come close to identifying the "true" G/NG boundary, and that the position of the boundary will move only slightly if an experimenter acts to control all of the above variables and to maximize the potential for the observation of growth in the chosen experimental system.

While the discussion has not focused specifically on appropriate methods for probability of growth within a defined time, many of the same principles and considerations will apply.

Moreover, the field of growth limits modeling, while having an equally long history as kinetic modeling, now seems to be quite disjointed, with little rigorous comparison of approaches, let alone agreement on the most appropriate model structures or experimental methods. In particular, the earlier work in probability modeling seems to have been ignored by some more contemporary workers, without reasons being indicated.

The results of G/NG studies are clearly of great interest to food producers and food safety managers. It is perhaps time, then, that the G/NG modeling community seeks to find common ground and to begin to develop a rigorous framework for the development, and interpretation, of growth limits studies.

APPENDIX A3.1 — CHARACTERIZATION OF ENVIRONMENTAL PARAMETERS AFFECTING MICROBIAL KINETICS IN FOODS

A3.1.1 Temperature

In most situations, temperature is the major environmental parameter in uencing kinetics of microorganisms in food and its effect is included in most predictive microbiology models. During processing, storage, and distribution the temperature of foods can vary substantially, frequently including periods of temperature abuse for chilled foods (see, e.g., Audits International, 1999; James and Evans, 1990; O'Brien, 1997; Sergelidis et al., 1997). Thus, it is an important property of secondary models that they can predict the effect of changing temperatures on microbial kinetics and application of these models relies on information about product temperature and its possible variation over time. Numerous types of thermometers, temperature probes, and data loggers are available (McMeekin et al*.,* 1993, pp. 257–269; seagrant.oregonstate.edu/extension/ sheng/loggers.html) to measure the temperature of foods or food processing equipment. Infrared non-contact thermometers are often appropriate for foods but their use is limited for process equipment with stainless surfaces.

A3.1.2 Storage Atmosphere

Foods are typically stored aerobically, vacuum packed, or by using modi ed atmosphere packing (MAP). "Controlled atmosphere packaging" can be considered a special case of MAP. MAP foods are exposed to an atmosphere different from both

air and vacuum packed usually involving mixtures of the gasses carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2).

O_2 and CO_2 in uence growth of most microorganisms and secondary predictive models must take their effect into account. The solubility of O_2 in water, and thereby into the water phase of foods, is low (~0.03 l/l) but it can be important for growth and metabolism of microorganisms in both aerobic and MAP-stored products (Dainty and Mackey, 1992). Numerous techniques and instruments are available to determine O_2 in the gas phase or dissolved in food. Microelectrodes to determine gradients of dissolved O_2 in foods are available (www.instechlabs.com/oxygen.html; www.microelectrodes.com/) but models to predict the effect of such gradients remain to be developed. To account for the effect of aerobic or vacuum packed storage of foods a categorical approach has been used within predictive microbiology. For aerobic conditions growth media with access to air have been agitated. For vacuum packed foods microorganisms typically have been grown under 100% N_2.

CO_2 inhibits growth of some microorganisms substantially and, to predict microbial growth in MAP foods, it is important to determine the equilibrium concentration in the gas phase or the concentration of CO_2 dissolved into the foods water phase. At equilibrium, the concentration of CO_2 dissolved into the water phase of foods is proportional to the partial pressure of CO_2 in the atmosphere surrounding the product. Henry's law (Equation A3.1) provides a good approximation for the solubility of CO_2.

$$\left[CO_2\right]_{aqueous}^{Equilibrium} = K_H \cdot \rho CO_2 \tag{A3.1}$$

In Equation A3.1, K_H is Henry's constant (mg/l/atm) and ρCO_2 is the partial pressure (atm) of CO_2. Between 0 and 160°C the temperature dependence of the Henry's constant can be predicted by Equation A3.2:

$$K_{\mathrm{H}}(mg \cdot l^{-1} \cdot atm^{-1}) = \frac{101325 \cdot 2.4429}{\exp(-6.8346 + 1.2817 \cdot 10^4 / K - 3.7668 \cdot 10^6 / K^2 + 2.997 \cdot 10^3 / K^3)} \tag{A3.2}$$

where K is the absolute temperature (Carroll et al., 1991). Those authors expressed K_{H} as MPa/mole fraction. In Equation A3.2 the constants 101,325 Pa/atm and 2.4429 was used to convert this unit into mg CO_2/l H_2O/atm.

For MAP foods in exible packaging the partial pressure of CO_2 is conveniently determined from the percentage of CO_2 inside the pack. A range of analytical methods is available to determine CO_2 concentration in gas mixtures or concentrations of dissolved CO_2 (Dixon and Kell, 1989; www.pbi-dansensor.com/Food.htm).

As shown from Equation A3.1 and Equation A3.2, the concentration of CO_2 dissolved in the water phase of a MAP food with 50% CO_2 in the headspace gas at equilibrium is 1.67 g/l at 0°C and 1.26 g/l at 8°C. Because of the high solubility of

CO_2 in water the gas composition in the headspace of MAP foods changes after packaging. The equilibrium gas composition is in uenced by several factors, e.g., the percentage of CO_2 in the initial headspace gas ($\%CO_2^{Initial}$), the initial gas/product volume ratio (G/P), temperature, pH, lipids in the food, respiration of the food, and, of course, permeability of the packing lm. Different mass-balance equations to predict the rate of adsorption and solubility of CO_2 have been suggested (Devlieghere et al., 1998; Dixon and Kell, 1989; Gill, 1988; Löwenadler and Rönner, 1994; Simpson et al., 2001a,b; Zhao et al., 1995). In chilled foods the rate of absorption of CO_2 is rapid compared to growth of microorganisms. Therefore, to predict microbial growth in these MAP foods it is suf cient to take into account the equilibrium concentration of CO_2.

Devlieghere et al. (1998) suggested Equation A3.3 to predict the concentration of CO_2 in the water phase as a function of $\%CO_2^{Initial}$ and G/P. In Equation A3.3, d_{CO2} is the density of CO_2 (1.976 g/l).

$$[CO_2]^{Equilibrium}_{aqueous} = \frac{\left(\frac{G}{P}\cdot dCO_2 + K_H\right) - \sqrt{\left(\frac{G}{P}\cdot dCO_2 + K_H\right)^2 - \left(\frac{4}{100}\cdot K_H \cdot \frac{G}{P}\cdot \%CO_2^{Initial}\cdot dCO_2\right)}}{2} \quad \text{(A3.3)}$$

Equation A3.3 does not take into account the effect of the storage temperature and Devlieghere et al. (1998) developed a polynomial model to predict the concentration of dissolved CO_2 as a function of $\%CO_2^{Initial}$, G/P, and temperature. If, for example, $\%CO_2^{Initial}$ is 25, the polynomial model predicts that a G/P ratio of three results in higher concentration of dissolved CO_2 than does a G/P ratio of 4, which is not logical. In contrast we have found that the combined use of Equation A3.2 and Equation A3.3 provides realistic predictions for concentrations of dissolved CO_2. It also seems relevant to include the effect of product pH on dissolved CO_2, and thereby the equilibrium concentration of CO_2 in the gas phase of MAP foods.

A3.1.3 Salt, Water-Phase Salt, and Water Activity

While temperature is the single most important *storage* condition in uencing growth of microorganisms in foods, NaCl is the most important *product* characteristic in many foods. The concentration of NaCl in foods can be determined as chloride by titration (Anon., 1995a). Instruments to determine NaCl indirectly from conductivity measurements are available but extensive calibration for particular types of products may be required. In fresh and intermediate moisture foods, NaCl is dissolved in the water phase of the products.

To predict the effect of NaCl on growth of microorganisms in these products the concentration of water-phase salt (WPS) or relative humidity, i.e., the water activity (a_w) must be determined (Equation A3.4 to Equation A3.7).

Water-phase salt can be calculated from Equation A3.4:

$$\text{\% Water phase salt} = \text{\%NaCl (w/v)} \times 100/(100 - \text{\% dry matter} + \text{\%NaCl (w/v)}) \quad \text{(A3.4)}$$

Water activity is a fundamental property of aqueous solutions and is de ned as:

$$a_w = \frac{\rho}{\rho_o} \quad \text{(A3.5)}$$

where ρ is the vapor pressure of the solution and ρ_0 is the vapor pressure of the pure water under the same conditions of temperature, etc.

For mixtures of NaCl and water there is a direct relation between the WPS content and a_w (Resnik and Chirife, 1988; Equation A3.6 and Equation A3.7). For cured foods where NaCl is the only major humectant these relations are valid as documented, e.g., for cold-smoked salmon (Jørgensen et al., 2000) and processed "delicatessen" meats (Ross and Shadbolt, 2001). To determine water activity of foods, instruments relying on the dew point method are now widely used because of their speed (providing results within a few minutes), robustness, and reliability but other methods and instruments are available (Mathlouthi, 2001).

$$a_w = 1 - 0.0052471 \cdot \%WPS - 0.00012206 \cdot \%WPS^2 \quad \text{(A3.6)}$$

$$\%\,WPS = 8 - 140.07 \cdot (a_w - 0.95) - 405.12 \cdot (a_w - 0.95)^2 \quad \text{(A3.7)}$$

A3.1.4 pH

For many microorganisms, small pH variations in the pH range ~6 to ~7 have very little or no effect on population kinetics. In more acidic foods, however, pH *per se* can greatly in uence microbial kinetics but can also accentuate the effect of other added preservative compounds. The pH of solid foods is often determined by homogenizing 10 g of a sample with 10 to 20 ml of distilled water and measuring the pH of the suspension using a standard combined electrode.

A3.1.5 Added Preservatives Including Organic Acids, Nitrate, and Spices

High concentrations of organic acids occur naturally in some foods and various organic acids including acetic acid, ascorbic acid, benzoic acid, citric acid, lactic acid, and sorbic acid are frequently added to foods. Organic acids can inhibit growth of microorganisms markedly and secondary models to predict their inhibitory effect are frequently needed. As for NaCl the secondary models must take into account the concentration of organic acids in the water phase of products. In addition, secondary models may need to describe the combined effect of organic acids and other environmental parameters particularly the pH.

In solution, organic acids exist either as the dissociated (ionized) or undissociated species. The Henderson–Hasselbalch equation (Equation A3.8) relates the proportion of undissociated and dissociated forms of organic acid to pH and pK_a according to the following expression:

$$[A^-]/[HA] = 10^{pH-pK_a} \quad (A3.8)$$

where [HA] is the concentration of undissociated form of the acid, [A$^-$] the concentration of dissociated (ionized) form of the acid, and pK_a is the pH at which the concentrations of the two forms are equal.

While both the dissociated and the undissociated forms of organic acids have inhibitory effects on bacterial growth the undissociated form is more inhibitory, usually by two to three orders of magnitude, than the dissociated form (Eklund, 1989).

Cross-multiplying and rearranging Equation A3.8 to solve for [HA] gives:

$$[HA] = [LAC]/(1 + 10^{pH-pK_a}) \quad (A3.9)$$

where [LAC] is the total lactic acid concentration and all other terms are as previously de ned.

As the concentration of an undissociated acid increases the growth rate of microorganisms decreases, eventually ceasing completely at a level described as the MIC. This behavior, and its dependence on the interaction of pH and total organic acid concentration, is included explicitly in several secondary models (Augustin and Carlier, 2000a; Presser et al., 1997).

Simple enzyme kits are available to determine several of the organic acids that are important in foods. Simultaneous determination of a range of organic acids is possible by HPLC analysis and is often an appropriate method to use (Dalgaard and Jørgensen, 2000; Pecina et al*.,* 1984).

Nitrite can be added to some types of meat products and its concentration in the water phase of products must be taken into account when secondary predictive models for these products are developed. Colorimetric methods are available to measure the concentration of nitrite in foods (Anon., 1995b; Karl, 1992).

Spices and herbs can have substantial antimicrobial activity and appropriate terms may need to be included in secondary models (Koutsoumanis et al., 1999; Skandamis and Nychas, 2000). The concentration of active antimicrobial components in spices, herbs, and essential oils can vary substantially as a function, e.g., of geographical region and season (Nychas and Tassou, 2000; Sofos et al., 1998). Therefore, the development of accurate secondary predictive models most likely will have to rely on the concentration of their active antimicrobial components. Recently, Lambert et al. (2001) showed the antimicrobial effect of the oregano essential oil quantitatively corresponded to the effect of its two active components, i.e., thymol and carvacrol. To quantitatively determine active components in spices, herbs, and essential oils appropriate extracts can be analyzed by GC/MS techniques (Cosentino et al., 1999; Cowan, 1999).

A3.1.6 Smoke Components

It has long been known that high concentrations of smoke components have strong antimicrobial activity (Shewan, 1949). Today many meat and seafood products are smoked but typically less intensively than some decades ago. However, even moderate concentrations of smoke components can in uence growth rates, growth limits, and rates of death/inactivation of microorganisms in foods (Leroi et al., 2000; Leroi and Joffraud, 2000; Ross et al., 2000b; Suñen, 1998; Thurette et al., 1998). Thus, to obtain accurate prediction of microbial kinetics in smoked foods it is important to include terms for the effect of smoke components in secondary models. Phenols are important antimicrobials in wood smoke, or in liquid smokes, and a few secondary models include the total phenol concentration as an environmental parameter (Augustin and Carlier 2000a,b; Giménez and Dalgaard, in press; Membré et al., 1997).

Classical colorimetric methods can be used to determine the total concentration of phenols in smoked foods. These methods rely on formation of colored complexes, e.g., between phenols and Gibb's reagent (2,6-dichloroquinone-4-chloroimide) or 4-aminoantipyrine (Leroi et al., 1998; Tucker, 1942). The total phenol concentration is a crude measure of how intensely foods have been smoked. By using GC/MS techniques more detailed information about specific smoke components can be obtained (Guillén and Errecalde, 2002; McGill et al., 1985; Tóth and Potthast, 1984). In the future, secondary models may be developed to include the effect of specific phenols, other specific smoke components, and possibly their interaction with NaCl. During the smoking of foods, phenols and other smoke components are mainly deposited in the outer 0.5 cm of the product (Chan et al., 1975). Modeling the effect of the spatial distribution in foods is another challenge.

A3.1.7 Other Environmental Parameters

The environmental parameters discussed above include those that are of major importance in traditional methods of food preservation. Many modern methods of food preservation also rely on combinations of these environmental parameters. However, the effect of a few well-known and several emerging food processing technologies relies on the antimicrobial effect of other environmental parameters, e.g., bacteriocins, gamma irradiation, high electric field pulses, high pressure, and UV light. Secondary models for the effect of some of these environmental parameters have been developed but will not be discussed here in detail. Other environmental parameters related to food structure and to the effect of microbial metabolism on changes in environmental parameters are discussed in Chapter 5 whereas the effect of time-varying environmental parameters is discussed in Chapter 7.

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